19 September 2013
EMA/586629/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Fluenz Tetra

Common name: influenza vaccine (live attenuated, nasal)

Procedure No. EMEA/H/C/002617/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

(att)	Attenuated
(ca)	Cold-adapted
CEK	Chicken Embryo Kidney cells
CPP	Critical Process Parameter
DS	Drug Substance
DP	Drug Product
EDTA	Ethylenediaminetetraacetic acid
ERA	Environmental Risk Assessment
FFA	Fluorescent Focus Assay
FFU	Fluorescent Focus Unit
GMO	Genetically Modified Organism
HA	Haemagglutinin
HAI	Haemagglutination Inhibition assay
IPC	In-process control
KOP	Key Operating Parameter
LAIV	Live attenuated influenza vaccine
M	Matrix protein
MDV	Master Donor Virus
MN	Microneutralisation
MVS	Master Virus Seed
NA	Neuraminidase Inhibition assay
NAI	Neuraminidase
NP	Nucleoprotein
NS	Non-structural protein
PA	Polymerase acidic protein
PB1	Polymerase basic protein 1
PB2	Polymerase basic protein 2
PCV	Porcine circovirus
PHF	Pooled harvest fluid
PhV	Pharmacovigilance
Q/LAIV	Quadrivalent live attenuated influenza vaccine
SPF	Specific Pathogen Free
TIV	Trivalent inactivated influenza vaccine
T/LAIV	Trivalent live attenuated influenza virus vaccine
(ts)	Temperature-sensitive
TSE	Transmissible spongiform encephalopathy
(wt)	Wild-type

1. Background information on the procedure

1.1. Submission of the dossier

The Applicant MedImmune, LLC submitted on 28 September 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Fluenz Tetra, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The Applicant applied for the following indication: Prophylaxis of influenza in individuals 24 months to less than 18 years of age. The use of Fluenz Tetra should be based on official recommendations.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The Applicant indicated that the live B/Yamagata strain in Fluenz Tetra was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on Applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision on the agreement of a paediatric investigation plan (PIP).

The PIP (P/0234/2012) was completed and the PDCO issued a positive opinion on full compliance check (EMEA-C-001051-PIP01-10-M03) on 14 June 2013.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the Applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The Applicant requested the live B/Yamagata strain contained in the above medicinal product to be considered as a new active substance in itself, as the Applicant claims that it is not a constituent of a product previously authorised within the Union

Scientific Advice

The Applicant received Scientific Advice from the CHMP on 16 December 2010 (EMA/CHMP/SAWP/784772/2010). The Scientific Advice pertained to clinical aspects of the dossier.

Licensing status

Fluenz Tetra has been given a Marketing Authorisation in the USA on 29 February 2012 (under the name FluMist Quadrivalent).

1.2. Manufacturers

Manufacturers of the biological active substance

MedImmune, UK Limited
Plot 6, Renaissance Way
Boulevard Industry Park
Speke
Liverpool L24 9JW
United Kingdom

Manufacturer responsible for batch release

MedImmune, UK Limited
Plot 6, Renaissance Way
Boulevard Industry Park
Speke
Liverpool L24 9JW
United Kingdom

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Daniel Brasseur Co-Rapporteur: Karsten Bruins Slot

- The application was received by the EMA on 28 September 2012.
- The procedure started on 24 October 2012.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 14 January 2013. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 12 January 2013.
- During the meeting on 21 February 2013, the CHMP agreed on the consolidated List of Questions to be sent to the Applicant. The final consolidated List of Questions was sent to the Applicant on 22 February 2013.
- The Applicant submitted the responses to the CHMP consolidated List of Questions on 22 May 2013.
- The final reports of the inspections carried out at MedImmune, LLC, 3055 Patrick Henry Drive, Santa Clara, CA 95054, USA on 26 April 2013 and at MedImmune, LLC, 297 North Bernardo Avenue, Mountain View, CA 94043, USA on 25 April 2013 were issued on 4 June 2013 and 5 June 2013, respectively.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant’s responses to the List of Questions to all CHMP members on 25 June 2013.

- During the CHMP meeting on 25 July 2013, the CHMP agreed on a list of outstanding issues to be addressed in writing by the Applicant.
- The Applicant submitted the responses to the CHMP List of Outstanding Issues on 15 August 2013.
- During a meeting of the Vaccine Working Party on 4 September 2013, experts were convened to address questions raised by the CHMP.
- During the meeting on 19 September 2013, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Fluenz Tetra.

2. Scientific discussion

2.1. Introduction

Influenza disease

Influenza is a highly contagious, acute febrile respiratory disease caused by one of two types of influenza viruses: influenza A and influenza B. As the circulating influenza strains may vary annually, epidemics occur on a yearly basis. The primary transmission of the disease is respiratory by means of large particle droplets. The incubation period generally ranges from 1-4 days, and viral shedding usually peaks around the second day of influenza symptoms. Children shed the greatest amount of virus and pose the greatest risk for further transmission of influenza into the community. Young children may shed virus for several days before the onset of symptoms and can be infectious for more than 10 additional days. Thus, infectivity is higher among preschool and school-aged children compared to other children and adults.

Type A influenza strains have been responsible for large epidemics. Since 1977, influenza A/H1N1, A/H3N2 and B viruses have circulated globally and have been included in all licensed influenza vaccines, as recommended by the World Health Organization (WHO). Influenza epidemics of variable severity occur annually worldwide in all age groups, typically during the winter months in temperate climates.

These annual epidemics are thought to result in 3 million to 5 million cases of severe illness and approximately 250,000 to 500,000 deaths every year around the world (WHO, 2005).

Influenza attack rates vary from year to year as do the circulating virus strains. The collaborative project European Influenza Surveillance Scheme (EISS) age-specific incidence rates reported have routinely been highest among those 0-4 and 5-14 years of age, though large variation was observed by countries (ECDC, 2007).

Influenza causes disease in all age groups. The clinical presentation of influenza in school-age children and adolescents is similar to that in adults and includes fever, cough, myalgia, headache, sore throat, chills tiredness and general malaise.

Uncomplicated influenza illness in healthy individuals is generally a self-limited febrile respiratory disease of 3-7 days' duration, sometimes with persistence of cough and malaise for several weeks. Influenza illness is characterized by the abrupt onset of signs and symptoms such as fever, myalgia, headache, malaise, chills, nonproductive cough, anorexia, sore throat, and rhinitis. Children may also

have otitis media, croup, nausea and vomiting. Severe cases can occur in children with underlying chronic diseases.

Severe morbidity and mortality occur mainly in the elderly (> 65 years of age) and the very young (< 24 months of age) and in other populations with specific “high-risk” conditions, such as chronic lung, heart, renal diseases or metabolic diseases, persons with conditions or medical treatments resulting in suppressed immune function, and persons living in institutional settings are at increased risk for influenza illness, development of serious influenza-associated complications (such as pneumonia and respiratory failure) and death.

Although influenza-associated deaths are uncommon among children (usually less than 100 per year in the United States but can be higher during years of vaccine mismatch), they represent a substantial proportion of vaccine-preventable deaths exceeding the annual child mortality from invasive pneumococcal disease, varicella, pertussis, or measles; 47% of the influenza-related deaths were in previously healthy children with no known risk factors or underlying chronic diseases.

The risk of influenza-associated hospitalization is greatest among the elderly (> 65 years old) and the very young (< 2 years of age). Infected children also appear to play a pivotal role in secondary transmission of influenza to household members and to other members of the community, leading to further increases in medical utilization and medication use.

Influenza virus

Two types are responsible for the disease: influenza A, which is categorized into subtypes on the basis of its haemagglutinin (H) and neuraminidase (N) surface antigens, and influenza B, which is separated into two genetic lineages.

Within each influenza subtype, the viruses undergo frequent changes in their surface antigens (antigenic drift), leading to the perpetuation of different viral strains (CDC, 2007).

A/H3N2 and A/H1N1 are the 2 influenza A subtypes that have circulated and caused human disease since 1977 (Kilbourne, 2006). Seasonal outbreaks in the last 2 decades have most commonly been associated with A/H3N2 strains. Influenza A/H3N2 strains have been associated with more severe illness and with higher mortality compared to seasons when A/H1N1 and B strains predominated (Simonsen et al, 1997; Thompson et al, 2003; Meijer et al, 2007).

Influenza seasonal vaccines

Vaccination is the most effective method for prevention of influenza (CDC, 2007). All current vaccines include antigens that can provide protection against influenza A and influenza B. Annual vaccination (1 or 2 doses depending on age and prior influenza vaccination history) aims at providing protection through an entire influenza season.

Each year, one or more strains contained in influenza vaccines might be changed to reflect the strains expected to circulate around the world. Since 1972, WHO has recommended 39 changes in the influenza vaccine formulation (WHO, 2005). Influenza vaccines must be administered annually to assure that populations are vaccinated with antigens that are relevant to circulating strains and provide optimal protection.

Trivalent seasonal influenza vaccines contain two A strains and one B strain. However, B strains from two antigenically distinct lineages, B/Victoria/02/87 and B/Yamagata/16/88, with limited immunological cross-reactivity, have been co-circulating annually, and the B strain selected annually

by public health authorities for inclusion in the seasonal influenza vaccine does not always match the predominant circulating B strains.

To address this issue, MedImmune has developed a quadrivalent influenza vaccine containing two A strains and two B strains (one from each of the 2 lineages).

Providing protection that is as broad as possible may be of particular importance for children and adolescents. Although influenza B causes disease in all age groups, its incidence relative to influenza A appears to be highest among older children and young adults. Furthermore, while influenza B causes mortality in all age groups, it appears to be a disproportionate cause of pediatric influenza deaths.

In summary, the development of a quadrivalent influenza vaccine represents in principle an advance over currently available trivalent influenza vaccines, as it expands the number of protective strains included in the vaccine, thereby increasing the likelihood of protection against circulating B strains of influenza.

2.2. Quality aspects

2.2.1. Introduction

Fluenz Tetra is a seasonal quadrivalent live attenuated influenza vaccine (Q/LAIV), developed as a successor to the currently authorised Fluenz seasonal trivalent live attenuated influenza vaccine (T/LAIV). The quadrivalent vaccine differs from the trivalent vaccine only in the number of influenza strains contained in the drug product.

Fluenz Tetra is presented in a refrigerated liquid formulation. It is to be administered intranasally, at the posology of 0.2 ml dose (0.1 ml per nostril). The drug product is produced by MedImmune, LLC (Philadelphia, Pennsylvania, USA), and imported and released in the European Union by MedImmune UK Limited (Speke, Liverpool, UK).

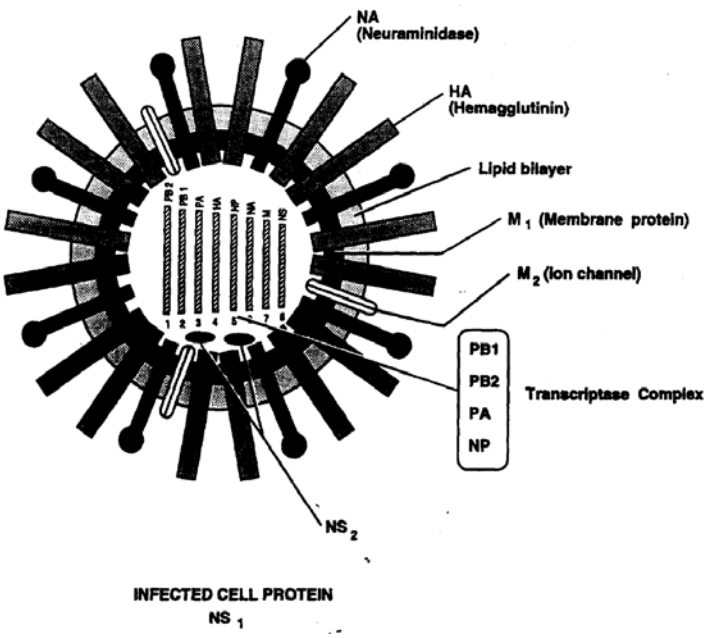
The drug substance consists in four different monovalent bulks of live attenuated influenza viruses (cold-adapted and heat sensitive), produced by MedImmune UK Limited (Speke, Liverpool UK). Monovalent bulks are prepared from purified harvests derived from the inoculation of the master virus seed (MVS) in embryonated Specific Pathogen Free (SPF) eggs. The master virus seeds (MVS) are prepared by a plasmid rescue process and contain a specific constellation of viral gene segments from an attenuated Master Donor Virus (MDV) and a wild-type (wt) influenza virus.

2.2.2. Active Substance

Influenza viruses Type A and Type B belong to the family of Orthomyxoviruses, and are morphologically indistinguishable from each other. Influenza viruses are enveloped and do not have a rigid capsule structure. The internal core of influenza virus particles consists of a segmented RNA genome, which is associated with the nucleoprotein <NP> and polymerase proteins. The viral envelope surrounds the viral nucleocapsid. The internal layer of the viral envelope contains viral matrix protein <M>, and the external layer of the envelope consists of a lipid bilayer that is derived from the host cell membrane during release of newly formed virus particles from infected cells. <NP> and <M1> proteins contain epitopes that provide the basis for antigenic distinction between Type A and Type B influenza viruses. The external surface of the lipid bilayer of influenza viruses is decorated with two major viral transmembrane protein spikes. Approximately 80% of these transmembrane protein spikes are rod-shaped haemagglutinin (HA) protein trimers, and 20% are mushroom-shaped neuraminidase (NA) tetramers.

The epidemiology of influenza viruses dictates incorporation of contemporary protective antigens (the haemagglutinin (HA) and neuraminidase (NA) antigens) into the vaccine on an annual basis. The HA protein is responsible for several of the biological properties of influenza viruses and the NA protein contributes to the antigenic characteristics and functional properties of influenza virus. Both the HA and NA protein epitopes contribute to the induction of a protective response in humans. Alterations in the primary structure of HA and NA proteins are directly related to antigenic variation of influenza viruses, which serves as the basis for antigenic and immunogenic characterization of influenza viruses using strain-specific antiserum.

Figure 1: Structure of the influenza A virus particle (Lamb, 1996)



Fluenz Tetra contains four active components: two attenuated influenza A strains, based on the influenza A master donor virus and two attenuated influenza B strains, based on the influenza B master donor virus. The cold-adapted reassortant vaccine strains in Fluenz Tetra are produced by genetic reassortment, using reverse genetics (plasmid rescue), between a wild-type influenza virus and cold-adapted master donor virus.

Such reassortant viruses contain gene segments encoding haemagglutinin (HA) and neuraminidase (NA) antigens that have been contributed by the wild-type virus, and gene segments encoding other proteins that have been contributed by the cold-adapted master virus (polymerase basic protein 1 <PB1>, polymerase basic protein 2 <PB2>, polymerase acidic protein <PA>, nucleoprotein <NP>, matrix protein <M>, and non-structural protein <NS>). These vaccine strains are called 6:2 reassortants.

Thus, cold-adapted reassortant vaccine strains derive their antigenic phenotypes from the wild-type strain and their cold-adapted (ca), temperature-sensitive (ts), and attenuated (att) phenotypes from the cold-adapted master donor virus.

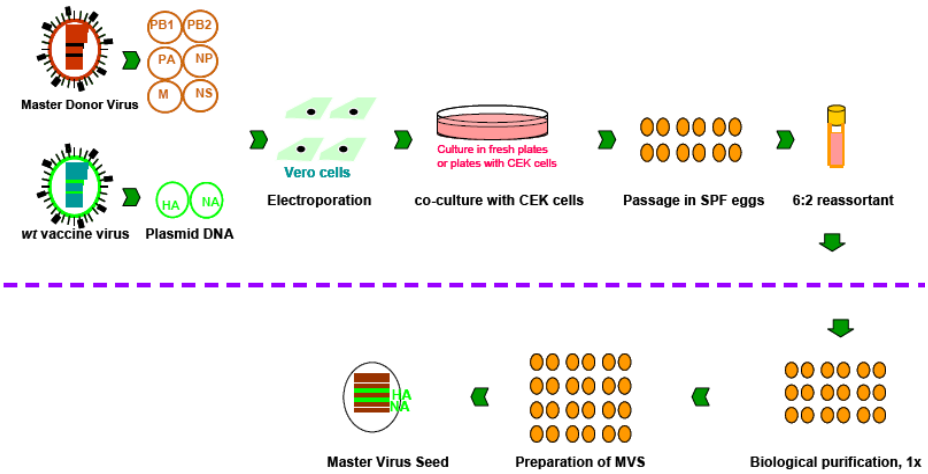
The two master donor virus strains used to make LAIV are A/Ann Arbor/6/60 and B/Ann Arbor/1/66 that were developed by H.F. Massaab (Massaab, 1982). The Type A and Type B ca master strains contribute internal viral proteins to reassortant Type A and Type B vaccines strains, respectively. The cold-adapted master strains possess characteristic phenotypic (ca, ts, att) markers that are linked to a genetically stable ca, ts, att phenotype.

The ca phenotype refers to the ability of the Type A or Type B cold-adapted master strain viruses to replicate to similar infectious titre in cell culture at either 33°C or 25°C. The ts phenotype of the Type A master strain viruses refers to the 39°C shut-off temperature of replication and the 100-fold or greater reduction in the number of plaques when compared to the permissive replication temperature of 33°C. The ts phenotype of the Type B ca master strain virus refers to the 37°C shut-off temperature of replication and the 100-fold or greater reduction in the number of plaques when compared to the permissive replication temperature of 33°C.

Manufacture of the Master Virus Seed (MVS)

The Master Virus Seeds (MVS) used in production consists in 6:2 reassortants containing a specific combination of six viral gene segments from an attenuated Master Donor Virus (MDV) and two gene segments contributed by a wild-type (wt) influenza vaccine virus encoding haemagglutinin (HA) and neuraminidase (NA) antigens. A new MVS is manufactured for each new influenza vaccine strain recommended by the World Health Organization (WHO). MVS are prepared by a plasmid rescue process / reverse genetics in Vero cells (which may use CEK cells as feeder cells).

Figure 2: MVS Manufacturing Process



The plasmid rescue process is initiated by extracting viral RNA from the MDV and the wt strain, and converting six viral gene segments (<PB1>, <PB2>, <PA>, <NP>, <M>, <NS>) from the MDV, and the HA and NA gene segments from the wt strain, into cDNA by Reverse Transcription Polymerase Chain Reaction (RT-PCR). These amplified cDNAs are inserted into plasmids and transformed into E. coli cells. The transformed E.coli cells are grown and plasmid DNA is purified for testing and further processing.

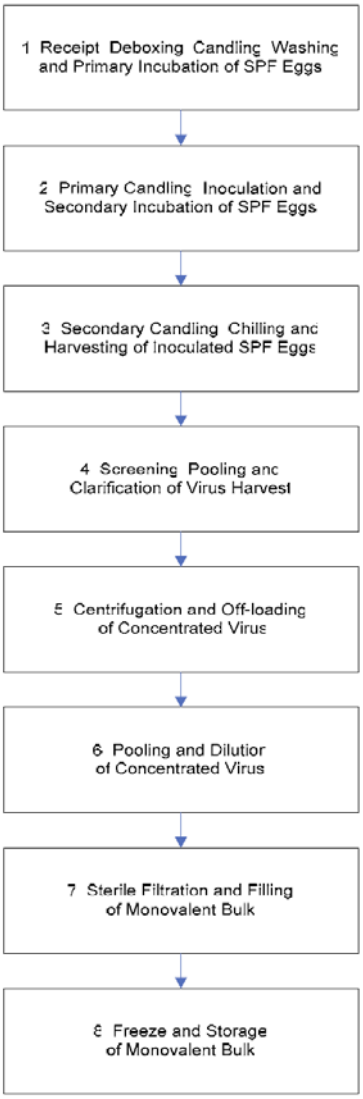
The cDNA containing plasmids corresponding to the MDV gene segment, as well as the cDNA containing plasmids corresponding to the wt HA and NA gene segments are combined by electroporation into serum-free Vero (African green monkey kidney) cells that are derived from an extensively tested and characterized cell bank produced in serum-free medium. The electroporated Vero cells are then either co-cultured with CEK cells, or alternatively, the electroporated Vero cells can be plated in the absence of CEK cells. Co-cultivation with CEK cells results in higher titres of virus. The 6:2 reassortants are then passaged in SPF embryonated chicken eggs to produce sufficient amount of material, this material is referred to as accession seed. An accession seed is made for each vaccine strain in the Research and Development Departments at the MedImmune CA facility. The accession seed is then transported to the MedImmune UK facility for further production of the MVS. The accession seed is biologically purified and amplified in SPF eggs to produce the MVS batch.

Each MVS is tested for sterility (bacterial/fungal contamination), mycoplasma and viral adventitious agents before release for further manufacturing.

Manufacture of the Monovalent Bulk Drug Substance

The drug substance is produced by MedImmune UK Limited (Speke, Liverpool UK). Monovalent bulks are prepared by the inoculation and growth of the master virus seed (MVS) in embryonated Specific Pathogen Free (SPF) eggs. After incubation of the eggs, the allantoic fluid that contains the vaccine virus is harvested. The bottles containing harvested fluid are sampled and placed in a cold storage area (2-8°C) prior to further processing. The pooled harvest fluid is filtered and centrifuged to concentrate the virus particles and to reduce the quantity of egg-derived proteins, nucleic acids and other components. The concentrated virus is diluted prior to sterile filtration. The resulting monovalent bulk is mixed and then dispensed into bottles using a closed system and stored at ≤ -60°C. The drug substance manufacturing process flow is shown in the figure hereafter.

Figure 3: Overview of the Monovalent Bulk manufacturing process



Receipt, preparation, incubation and inoculation of Specific Pathogen Free (SPF) Eggs

Upon shipments SPF eggs are transferred to refrigerated storage and checked for compliance with predefined specifications and integrity. Eggs are washed, rinsed, dried and transferred into the Primary Incubation suite.

Following the primary incubation period, the trolleys of eggs are removed from the primary incubators and transferred to the Primary Candling Area where they are candled for any cracks etc. The trays of acceptable candled eggs are transferred and held under controlled temperature conditions until inoculation. The eggs are inoculated with diluted Master Virus Seed (MVS) using a peristaltic pump and incubated. After incubation, allantoic fluid is removed. Clear allantoic fluid is dispensed into a sterile polycarbonate bottle and transferred to the Harvest Cold Room until released for further processing.

Screening, Pooling and centrifugation of Virus Harvest

Harvest bottles are screened for lack of bioburden using a rapid bioburden screening assay and pooled. The pooled and mixed fluid is then sampled to provide material for safety testing. The pooled material is filtered to obtain Clarified Harvest Fluid (CHF). The CHF is mixed and samples are removed for bioburden in-process testing. Pooled virus harvest is concentrated using continuous flow ultracentrifugation in a sucrose gradient in order to increase the density of virus particles present in

the CHF and to reduce the quantity of egg-derived proteins, nucleic acids and other components. Concentrated virus harvest is pooled and pooled fractions are then diluted using phosphate buffer/EDTA prior to sterile filtration.

Sterile Filtration and Filling

The diluted virus harvest pool is sterile filtered to obtain the monovalent bulk. Following flushing and equilibration, the sterilizing grade filter is subjected to a filter integrity test.

In-Process Manufacturing Controls

Critical Process Parameters (CPP) and Key Operating Parameters (KOP) were defined for the manufacturing of the monovalent bulk DS, based on their ability to impact on Critical Quality Attributes (CQAs) and process performance, respectively.

In-process control (IPC) limits have been established for key process parameters that are used to monitor ongoing production of the monovalent bulk DS.

Process validation

Process validation studies were conducted in support of the initial monovalent bulk DS manufacturing process and changes introduced since then.

The study results demonstrated that the manufacturing process is capable to consistently yield monovalent bulk DS that is compliant with the specifications.

Control of materials – materials of biological origin

Animal-derived components used in the DS manufacturing process are: SPF Hen's eggs, Vero cells and CEK cells, porcine trypsin, fetal bovine serum and the newborn calf serum.

Manufacturing process development

The DS material used in the pivotal Q/LAIV clinical trials were not DS lots produced by the final commercial process. Several changes have been implemented in the DS manufacturing process after production of the clinical DS material. However, these changes were properly validated and demonstrated to have no impact on the quality of the monovalent bulk DS. Characterization studies indicate that the quality of the materials used in the clinical studies and the quality of batches produced with the commercial process are comparable.

Specification

Characterisation

The characterisation studies of the drug substance have been presented as a summary of the different comparability exercises and led to the analysis of attenuation and phenotype (ca/ts), maintenance of consensus DNA sequence from the respective MVS, virus particle morphology, percent infectious particles, HAI, HA and Viral RNA content. Replication and immunogenicity studies and protection following wild type challenge were performed in ferrets.

Impurities

The impurities identified by the Applicant are egg related components in the allantoic fluid (ovalbumin, protein, and chicken DNA) and other process related impurities (Disodium EDTA and Gentamicin Sulfate).

Upon harvesting of the allantoic fluid and subsequent pooling, measurable levels of ovalbumin, total protein, and DNA can be found in the Pooled Harvest Fluid (PHF). During the downstream processing,

particularly at the ultracentrifugation step, significant removal of these egg related impurities takes place. Most of the ovalbumin is consistently removed during the manufacturing process. Nearly all of the total proteins are removed during processing of monovalent bulk DS. Residual DNA is also reduced to sufficiently low levels. Ovalbumin, total protein, and DNA are routinely monitored by in-process testing on the filtered monovalent bulk DS.

During the manufacture of the monovalent bulk, the H3N2 serotype is diluted using phosphate buffer containing Disodium EDTA. The concentration of EDTA in the drug product is a release specification.

Gentamicin Sulfate is a component of the media used in the MVS manufacturing process. The theoretical concentration of Gentamicin Sulfate in the final product is below levels of detection using current assay methods.

Control of Drug Substance

Drug substance release tests are performed on samples collected at the Pooled Harvest Fluid (PHF) and monovalent bulk DS stages. The DS lot release specifications for LAIV are identical for all vaccine strains and are in accordance with the Ph.Eur. requirements where applicable.
Bioburden, absence of selective organisms, in vivo and in vitro adventitious viral agents, mycoplasma, reverse transcriptase and avian leukosis virus tests are performed on the PHF, while sterility, endotoxin, appearance, potency (Fluorescent Focus Assay), genotype (6:2 reassortant), phenotype (*ca* & *ts*), attenuation, and identity by HAI tests are performed on the monovalent bulk DS.

The different analytical procedures were described in detail and adequate validation reports and studies were provided. The proposed specifications are deemed acceptable.

The Applicant has presented batch analysis data from bulks used to manufacture Fluenz Tetra process validation batches, batch analysis data for Fluenz batches manufactured for the 2012-2013 season as well and batch analysis data for Fluenz process validation supporting the current manufacturing process. All presented data is within the pre-set acceptance criteria.

Container closure system

The monovalent bulk drug substance is filled into a polycarbonate (PC) bottle, sealed with a polypropylene screw-on cap containing a silicon rubber liner. The container closure system, as well as the studies on extractables and leachables, was deemed appropriate.

Stability

The shelf life of the LAIV monovalent bulks is 24 months stored at ≤ -60°C.
The shelf life is supported by stability studies performed throughout the product development for LAIV. This includes the trivalent formulation of live attenuated influenza vaccine (T/LAIV) batches that have been distributed commercially. This is because the production process for the monovalent bulk drug substance used for manufacture of Q/LAIV is the same as for that used for production of the authorised trivalent vaccine T/LAIV.
Test methods for stability are test for potency and sterility. Extensive stability data available from each season's manufacturing campaign at production scale support the proposed shelf life.

2.2.3. Finished Medicinal Product

The finished product is a sterile colourless to pale yellow liquid composed of four serotypes (A/H3N2, A/H1N1, B/Yamagata and B/Victoria), and formulated with monosodium glutamate, gelatin, arginine, sucrose, and phosphate buffer. The finished product is presented as a 0.2 ml nasal sprayer capable of

delivering a dose of 7.0 ± 0.5 $\log_{10}$ FFU of each strain and 0.1 ml is sprayed in each nostril. The composition of Fluenz Tetra drug product is included in table 1.

Table 1: Composition of Fluenz Tetra

Components	Quality Standard	Function
Active Components		
Influenza Virus, Type A, H1N1	MedImmune	Immunogen
Influenza Virus, Type A, H3N2		Immunogen
Influenza Virus, Type B (Yamagata lineage)		Immunogen
Influenza Virus, Type B (Victoria lineage)		Immunogen
Inactive Components		
Sucrose	Ph.Eur./NF	Stabilizer
Dipotassium Phosphate (Dibasic potassium phosphate) [b]	Ph.Eur./USP	Buffer
Potassium Dihydrogen Phosphate (Monobasic potassium phosphate) [b]	Ph.Eur./NF	Buffer
Gelatin Hydrosylate, Porcine Type A [b]	Ph.Eur./NF	Stabilizer
Arginine hydrochloride	Ph.Eur./USP	Stabilizer
Monosodium Glutamate Monohydrate	Ph.Eur./NF	Stabilizer
Water for Injection	Ph.Eur./USP	Solvent

The finished product is produced by MedImmune, LLC, Philadelphia, PA, USA, and is released in the EU by MedImmune UK Limited, Speke, Liverpool, UK.

Pharmaceutical Development

Formulation development

The quadrivalent live attenuated influenza vaccine (Q/LAIV) vaccine formulation was established based on the trivalent (T/LAIV) formulation and was chosen for suitability for intranasal administration.

The T/LAIV drug product formulation has been shown to be safe and well tolerated based on extensive clinical studies and global marketing experience. A comparison of the T/LAIV and Q/LAIV formulations demonstrates that the chosen excipients and their concentrations are identical between T/LAIV and Q/LAIV.

Altogether, 1 Q/LAIV clinical batch (used in the pivotal safety and immunogenicity clinical studies) and 3 process validation batches using the current formulation have been successfully produced for the Q/LAIV MAA filing for demonstration of the consistency of the Q/LAIV manufacturing process.

Manufacturing process development

The manufacturing process for the Q/LAIV clinical batch was based on the T/LAIV commercial manufacturing process at the time. The Q/LAIV clinical lot was used in the controlled pivotal safety and immunogenicity clinical studies for Q/LAIV.

No process development studies specific for Q/LAIV have been performed because the only differences between the Q/LAIV and T/LAIV process is the addition of the fourth strain at the blending step. This

change did not require any additional in-process testing or modification of the process parameters at the blending step.

The specification used for release of the Q/LAIV drug product are identical to those that release the commercial T/LAIV, except that the Total Potency specification of ≤ 8.0 Log_{10}FFU/dose was introduced as a new specification for Q/LAIV to ensure that the calculated total viral potency of vaccine strains for Q/LAIV does not exceed the total viral potency for the T/LAIV.

Manufacture of the product

The manufacturing process mainly consists in the thawing of the monovalent bulks, followed by a blending of the 4 DS monovalent bulks to final volume with buffers.

The blended quadrivalent formulated bulk is aseptically filled as a 0.2 ml deliverable dose into 0.5 ml Accuspray nasal sprayer barrels, without any additional sterilisation step. The product is frozen at ≤ -20°C prior to or after final packaging (with secondary labelling). The product is then transported to a warehouse prior to shipment. In the EU, the product is batch released by MedImmune UK Ltd, Speke, UK.

The quantities of each component blended together yield the final quadrivalent bulk formulation. The quadrivalent bulk formulation calculations are based upon the actual release titre results from the four monovalent virus strain lots to be used in the final quadrivalent bulk formulation.

In-Process Manufacturing Controls

Critical steps in the manufacture of the drug product are controlled at several steps in both the blending and filling processes to ensure that the process performs as intended.

Process validation

Validation of final drug product blending and filling process at the MedImmune Pennsylvania facility was performed on three batches of H1N1 vaccine. The results showed that the acceptance criteria for the thawing, blending and filling processes were met.

Specific Q/LAIV process validation studies were designed to provide assurance that the specific manufacturing processes would consistently produce a product meeting its predetermined specifications and product quality attributes. Three process validation batches were analysed and all test results met the specifications.

Adventitious agents

The master virus seeds (MVS) are prepared by a plasmid rescue process and contain a specific constellation of viral gene segments from an attenuated Master Donor Virus (MDV) and a wild-type (wt) influenza virus. Vero cells are electroporated with plasmids containing cDNA clones of the viral gene segment and then co-cultured with SPF CEK cells to produce pre-MVS/MVS. Raw materials of animal origin (FBS, NCS, porcine trypsin) were used for Vero cell banks establishment and during cultures of CEK cells.

The MVS are used to inoculate Specific Pathogen Free (SPF) embryonated eggs to produce individual monovalent bulks for each of the four virus strains.

The viral safety relies on i) quality/virological controls of raw materials of animal origin used during the process and ii) virological controls performed on cell substrates (Vero and CEK cells), SPF eggs and during production process at MVS level and pooled harvest fluid level.

TSE

In accordance with the Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01), Certificates of suitability for TSE safety have been provided for all raw materials derived from TSE relevant animal species used in the manufacturing process.

Overall, sufficient data is provided to exclude a risk of TSE transmission through Fluenz Tetra. The risk of transmitting TSE by Fluenz Tetra is thus considered very remote.

Product specification

Control of Drug Product

The drug product specification consists in identity, potency on each individual strain, total potency, pH, endotoxin, ovalbumin, total protein, colour, opalescence and appearance, sterility, EDTA and thermal stability. The identity and potency tests are based on the Fluorescent Focus Assay (FFA assay), while total potency is determined by calculation.

Sterility is also performed on the bulk Q/LAIV blend.

Descriptions of the analytical methods as well as the validation studies for non compendial methods were provided.

Container closure system

The container/closure system for the quadrivalent live, attenuated influenza vaccine (Q/LAIV) drug product is the Accuspray Nasal Spray System. The nasal spray system consists of a nasal sprayer barrel, Nasal Sprayer Nozzle, Plunger Stopper and Plunger Rod.

Stability of the product

Stability data for 3 batches of Fluenz Tetra have been presented. The presented data covered a period of 12 weeks at -25°C ± 5°C and up to 32 weeks subsequent storage at 2°C to 8°C. Additional data covering the whole shelf life will be submitted upon completion of the Q/LAIV stability study.

The proposed shelf life for Fluenz Tetra (Q/LAIV) is identical to the shelf life approved for Fluenz (T/LAIV). As the addition of a fourth strain is not expected to significantly change the quality characteristics of the Q/LAIV, stability data of the T/LAIV were considered supportive for the Q/LAIV.

The Drug Product stability data support the shelf-life at -25°C ± 5°C for up to 20 weeks prior to distribution and subsequent storage at 2°C to 8°C not to exceed 18 weeks.

In accordance with EU GMP guidelines, any confirmed out of specification result, or significant negative trend, should be reported to the CHMP.

GMO

Like Fluenz (T/LAIV), Fluenz Tetra (Q/LAIV) is a live attenuated influenza vaccine containing virus strains generated through reverse genetic technology. For this reason, the vaccine is classified a genetically modified organism (GMO) as defined in Directive 2001/18/EC.

Fluenz Tetra contains two types A (i.e. A/H1N1 and H3N2) and two type B (i.e. B/Victoria and B/Yamagata) attenuated (att), cold-adapted (ca) and temperature sensitive (ts) reassortant strains. Each dose is formulated to contain $10^{7\pm0.5}$ fluorescent focus units (FFU) of each of the four reassortant influenza virus strains.

The plasmid rescue process utilises recombinant DNA techniques to produce genetic reassortants. Each of the four vaccine strains are 6:2 genetic reassortants. These vaccine strains have 6 gene segments

(<PB1>, <PB2>, <PA>, <NP>, <M> and <NS>) from one master donor viruses (MDV, type A or B) and 2 gene segments, haemagglutinin (HA) and neuraminidase (NA) from the WHO recommended contemporary wt influenza virus.

The specific genotype of MVDs is designated 6:2 which indicates that 6 internal gene segments that confer the characteristics of the ca, ts and att phenotypes are derived from the MDV and that the 2 gene segments encoding HA and NA surface antigens are derived from the wt influenza strains.

The environmental risk was evaluated for Fluenz T/LAIV as part of the application for marketing authorisation.

Fluenz does not replicate freely in the environment. It is specific to humans and a few mammalian species (hamsters, guinea pigs and ferrets are known to be capable of being experimentally infected with human influenza virus). The Fluenz vaccines do not carry a toxic transgene, do not integrate and therefore are very unlikely to transfer genes to any other species. The overall risk posed by this GMO to human health and the environment was considered low or negligible.

The initial environmental risk assessment (ERA) was considered to remain relevant for future seasonal strains. Submission of an ERA at each seasonal strain update procedure was not required, with all reserves of new scientific information publication on the ERA for this kind of vaccine.

The addition of a fourth strain does not change the risk to the environment. The manufacturing procedures are identical and the genetic stability is comparable.

The CHMP concludes that the overall risk to the environment from Fluenz Tetra is low.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The Applicant has provided detailed information on the DS manufacturing process, including a description of all the starting materials and of the production of the Master Virus Seeds using the reassortment plasmid rescue technique. All steps involved in the manufacturing process were adequately validated.

The genetic stability of each new MVS is evaluated by passaging the MVS for four passages beyond production level (i.e., MVS+5 passages), and then sequencing the final passage material to demonstrate genetic stability. If any amino acid changes are observed between the MVS and MVS+5 isolates in any of the loci known to affect the (ca), (ts) or (att) phenotypes, phenotype and attenuation tests are performed to determine the stability of the (ca), (ts) and (att) phenotypes. The Applicant will document and justify any mutation between the MVS and MVS+5 isolates in the annual strain update variation.

The Applicant has confirmed during the procedure that the gamma-irradiated porcine trypsine, used for the manufacture of Master Virus Seeds, is tested for porcine circovirus (PCV) and that the gelatin production process contains virus inactivation steps that are able to inactivate PCV.

The DS material used in the pivotal Q/LAIV clinical trials were not DS lots produced by the final commercial process and several changes have been implemented in the process after production of the clinical material. However, these changes were demonstrated to have no impact on the quality of the monovalent bulk DS. The overall results of the analytical studies performed indicated that the quality of the materials used in the clinical studies and the quality of batches produced with the commercial process are comparable.

The different analytical procedures were described in detail and adequate validation reports and studies were provided. The Haemagglutination - Inhibition assay (HAI assay), performed at release on

monovalent bulks, was validated using two A strains (H1N1 and H3N2) but only one B strain. The Applicant has committed to re-qualify the assay using two B strains representing the two different B lineages, prior to distribution of the product.

The proposed DS specifications were sufficiently justified and are deemed acceptable. The limits for bioburden and endotoxin, which seemed high, have been appropriately justified. Likewise, the specification limits for ovalbumin have been satisfactorily justified, and the Applicant will establish an action/alert limit.

The container closure system was deemed appropriate as well as the studies on extractables and leachables. The proposed DS storage time and conditions of 24 months stored at ≤ -60°C was sufficiently substantiated.

The development of the manufacturing process (formulation) of the drug product has been described in detail and the different process changes were highlighted. Validation of the DP manufacturing process was appropriately performed.

Descriptions of the analytical methods as well as the validation studies were provided. The proposed specifications for the DP were sufficiently justified and are deemed acceptable.

As compared to Fluenz (T/LAIV), a new Fluorescent Focus Assay to determine potency had initially been proposed. A modified procedure is under development and will be submitted once validated. In addition, the Applicant will provide results from stability tests performed with the modified potency assay upon availability.

The proposed container closure system for the final product is acceptable and sufficient data supporting the suitability of the Accuspray Nasal Spray System were provided.

Finally, the shelf life claim of the drug product at "-25°C ± 5°C for up to 20 weeks prior to distribution and subsequent storage at 2°C to 8°C not to exceed 18 weeks" is deemed acceptable based on the supportive information that was provided.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Based on the review of the data on quality, the manufacture and control of the Fluenz Tetra drug substance and the drug product are considered acceptable.

The Quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in satisfactory way.

Safety concerning adventitious agents including TSE has been sufficiently assured.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

Description of post-authorisation measures
1. As regards the evaluation of the genetic stability of the MVS, the Applicant is recommended to document and justify any mutation case by case in the variations for annual strain update.
2. The Applicant is recommended to re-qualify the HAI assay using two B strains representing the two different lineages.

Description of post-authorisation measures
3. The Applicant is recommended to establish an action limit for ovalbumin (at the level of the Drug Product). 4. The Applicant is recommended to continue stability tests performed with the modified potency assay. For these stability studies it is not deemed necessary to include all time points as performed in the original MAA file. A reduced testing scheme (with less time points, but which allows calculation of degradation slope) is acceptable (the 18 weeks' time point (at 2-8°C), however, should be included).

2.3. Non-clinical aspects

2.3.1. Introduction

Fluenz Tetra and Fluenz are manufactured in an identical manner, the viral content per strain and the inactive components of Fluenz Tetra and Fluenz are identical. In addition, both vaccines are administered intranasally by the same delivery device.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Immunogenicity and challenge studies of different Fluenz and Fluenz Tetra formulations in ferrets

Pharmacologic properties of frozen and refrigerated formulations of Fluenz were tested in ferrets. This study showed that the performance of the tested refrigerated and frozen formulations were similar with respect to vaccine take, replication, immune response induction, and protection of animals from a challenge infection with wild type virus.

Immunogenicity and potential immune interference were tested in ferrets with 2 different vaccine viruses of type B in the formulation. Three different combinations of 2 vaccine viruses of 2 types B (1 from B/Yamagata and 1 from B/Victoria lineages), either in bivalent or monovalent formulations, were evaluated. The addition of a second vaccine virus of different a type B lineage to the vaccine did not cause immune interference.

Immunogenicity and protection following challenge with the wild-type (wt) influenza viruses after vaccination with Q/LAIV and Fluenz were evaluated in ferrets in 4 studies. Q/LAIV was immunogenic in ferrets. Both the Q/LAIV vaccine and the corresponding trivalent vaccine formulations reduce the amount of wild type viruses in the respiratory tract as compared to the amount of virus detected in placebo ferrets.

Immunogenicity studies of Fluenz Tetra in rats

A repeat-dose immunogenicity study was done in female rats. Q/LAIV was immunogenic in non-pregnant female rats after the second dose.

A repeat-dose immunogenicity study was done in pregnant females and their live-born offspring. In non-primed pregnant rats, a robust immune response to the Q/LAIV is only elicited after 3 administrations and by gestation day 21, which is 1 day before birth. In the design of the rat reproductive toxicity study, a group where the rats were primed before mating has been included.

The immune response of the offspring is a bit lower than that in mothers. Little variation of HAI titre was observed between offspring siblings.

Secondary pharmacodynamic studies

Secondary pharmacodynamic studies are generally not performed with vaccines and were not performed with Fluenz Tetra. As the vaccine did not show any effects apart from the expected immune response, this was considered acceptable.

Safety pharmacology programme

Neurovirulence testing of influenza strains in mice

The results of the neurovirulence study in mice submitted for the Fluenz application can support the Fluenz Tetra Q/LAIV application and indicate that the Q/LAIV viruses do not exhibit any neurotropism or neurovirulence.

For annual strain updates for Fluenz, the Applicant agreed to conduct a neurovirulence assay on the master virus strains of any novel strain subtype(s) introduced into the vaccine that have not been previously tested. In addition, a control strategy for monitoring neurovirulence of any novel strain subtypes was developed, to take advantage of several surveillance and reporting methods already in place including post-marketing safety data, periodic safety update report and risk management plan.

The same commitments as for Fluenz with respect to neurovirulence assays and neurovirulence monitoring are agreed for Fluenz Tetra.

Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies have not been conducted with Fluenz Tetra in accordance with “Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines” (CPMP/SWP/465/95) and with “Guideline on Adjuvants in Vaccines for human use” (CHMP/VEG/134716/2004).

Clinical studies were performed with Fluenz to evaluate safety, tolerability and immunogenicity of the vaccine administered concurrently with measles, mumps, rubella (MMR), varicella and oral polio vaccines in young children. The safety and immunogenicity profiles of the respective vaccines (Fluenz, MMR, VAR, and OPV) were not altered when administered concomitantly vs. separate administration.

2.3.3. Pharmacokinetics

While many of the typical pharmacokinetic studies, including absorption, metabolism and excretion, do not pertain to live vaccines, local deposition and distribution studies have been performed in humans. The vaccine does not contain an adjuvant or new excipients which would require other pharmacokinetics studies.

2.3.4. Toxicology

Fluenz Tetra and Fluenz are manufactured in an identical manner, the viral content per strain and the inactive components are identical. In addition, both vaccines are administered IN by a delivery device. Pharmacodynamic studies with Q/LAIV in rats and ferrets revealed no different pharmacologic properties when compared to Fluenz. Thus, toxicology studies conducted with Fluenz can support Q/LAIV.

In addition, two new GLP-compliant toxicology studies were conducted to support the safety of Q/LAIV (a repeat-dose toxicity study in ferrets and a reproductive and developmental toxicity study in rats).

Single dose toxicity

A single dose toxicity study was incorporated into the repeat dose toxicity study.

Repeat dose toxicity

Two repeat-dose toxicity studies were conducted in ferrets: 1 with Fluenz Tetra Q/LAIV (Study SVT08-18) and 1 with Fluenz (Study SVT01-18). These studies also evaluated single-dose toxicity and local tolerance of the vaccine. Repeat-dose toxicity studies with Fluenz Tetra and Fluenz showed no significant systemic or local toxicity.

Genotoxicity

No genotoxicity study was submitted for Fluenz Tetra in accordance with the CHMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95).

Carcinogenicity

No carcinogenicity study was submitted for Fluenz Tetra as recommended by the CHMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95).

Reproduction Toxicity

Reproductive and developmental toxicity of Fluenz Tetra and Fluenz in rats were investigated in Study 20001854 and Study 3113-001, respectively. In addition, developmental toxicity of Fluenz was investigated in ferrets in Study SVT01-19. Fluenz Tetra and Fluenz did not affect reproduction and development in rats; F0 generation females and F1 generation offspring were unaffected by either vaccine.

Toxicokinetic data

Not applicable.

Local Tolerance

Evaluation of local tolerance at the administration site is included in the repeated dose toxicity studies in ferrets with the evaluation of the nasal mucosa.

The potential for ocular toxicity resulting from the inadvertent instillation of Fluenz into the eye was evaluated in two ocular toxicity studies in rabbits. A standard Draize test was performed in two separate studies using the frozen and refrigerated formulations of Fluenz. Neither study elicited results consistent with ocular toxicity.

Other toxicity studies

No specific studies were performed.

2.3.5. Ecotoxicity / environmental risk assessment

Fluenz Tetra, like Fluenz/FluMist, contains live attenuated viruses which are prepared by reverse genetics techniques, therefore these are considered GMOs. Environmental safety studies with Fluenz designed to evaluate the tropism of the vaccine for non-human species were conducted in 21 animal species, as the vaccine virus does not replicate freely in the environment. Replication of vaccine viruses was measured in respiratory tissues of the animals. The vaccine viruses did not replicate in any bird species. In mammals, replication of the vaccine viruses was only noted in hamsters, guinea pigs, and ferrets. These species have been shown previously to be experimentally infected with human influenza virus. Thus, the vaccine viruses did not gain novel tropism for nonhuman species. In addition

Fluenz T/Q does not carry a toxic transgene and does not integrate; therefore genes transfer to other species is very unlikely. The overall risk posed by this GMO to human health and the environment is considered low or negligible.

The CHMP concluded that the ERA performed for the initial MAA of Fluenz remains relevant for Fluenz Tetra, since the addition of a fourth strain does not change the risk for the environment. The manufacturing procedures are identical and the genetic stability is comparable between the two vaccines. It also remains relevant for future seasonal strain updates. Submission of a new ERA at the time of subsequent seasonal strain updates is not required, with all due reserves in case of new scientific information published on this topic.

2.3.6. Conclusion on non-clinical aspects

Non-clinical data with Fluenz Tetra revealed no specific concerns for humans based on conventional non-clinical studies for repeated dose toxicity, reproduction and developmental toxicity, local tolerance and neurovirulence. The CHMP considers that no additional measures are necessary.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the Applicant.

The Applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies:

Study No. Country	Study description	Study Start/End	Vaccination schedule	Immunizations	Number of subjects vaccinated
MI-CP185 United States	Phase 2b/3 immunogenicity and safety study Double-blind, active control Randomization 4:1:1 Evaluation of non-inferiority of Q/LAIV to Fluenz/B/Yamagata and Fluenz/B/Victoria	23 March 2009/9 Oct 2009	1 dose (Day 0) BD Accuspray Healthy subjects 18-49 years	Q/LAIV 0.2 ml IN	1197
				Fluenz-Y 0.2 ml IN	298
				Fluenz-V 0.2 ml IN	299
MI-CP206 United States	Phase 2b/3 immunogenicity and safety study Partial-blind, active control Randomization 4:1:1 Evaluation of non-inferiority of Q/LAIV to Fluenz/B/Yamagata and Fluenz/B/Victoria	14 August 2009/3 March 2010	1 dose (Day 0) Blow-fill-seal Healthy subjects 18-49 years	Q/LAIV 0.2 ml IN	1198
				Fluenz-Y 0.2 ml IN	298
				Fluenz-V 0.2 ml IN	298
MI-CP208 United States	Phase 2b/3 immunogenicity and safety study Partial-blind, active control Randomization 3:1:1 Evaluation of non-inferiority of Q/LAIV to Fluenz/B/Yamagata and Fluenz/B/Victoria	29 March 2010/27 December 2010	2-8 years: 2 doses (Day 0/Day 28-35) 9-17 years: 1 dose (Day 0) BD Accuspray Healthy subjects 2-17 years	Q/LAIV 0.2 ml IN	1382
				Fluenz-Y 0.2 ml IN	463
				Fluenz-V 0.2 ml IN	460

IN: intranasal

- Tabular overview of analytical methods used in the paediatric studies of the Fluenz Tetra (Q/LAIV) and Fluenz clinical development programmes:

Clinical studies	Analytical method
Q/LAIV and Fluenz	Serum hemagglutination inhibition assay
	Microneutralization assay
Q/LAIV	Neuraminidase inhibition assay
Fluenz	Median tissue culture infectious dose (TCID50) assay
	Assessment of viral shedding
	Serum IgG and nasal IgA enzyme-linked immunosorbent assays
	Phenotyping assay
	Genotyping/subtyping assay
	Hemagglutination inhibition assay for antigenic identification
	Sequencing

2.4.2. Clinical Pharmacology

Fluenz Tetra is a live, attenuated virus vaccine composed of 4 reassortant influenza viruses that replicate locally in the mucosa of the upper respiratory tract and induce both localized and systemic immune responses. Since classical pharmacology studies do not pertain to this type of product, clinical pharmacokinetics studies have characterised the in vivo deposition and distribution of intranasally administered vaccine vehicle and clinical pharmacodynamics studies should investigate vaccine-induced immune responses.

Pharmacokinetics

These studies aimed at characterizing the in vivo deposition and distribution of intranasally administered vaccine vehicle.

The initial deposition and clearance of frozen and refrigerated vehicle (i.e. excipient only) formulations of Fluenz were evaluated in a randomized, open-label, 2-way crossover study in 21 adults (Scintigraphy Study PPL-1014). Vehicle formulations were mixed with the radio-labelled marker prior to intranasal administration via the Accuspray™ device, and in vivo distribution was determined using standard scintigraphy nuclear imaging.

Intranasal delivery of 0.2 ml refrigerated vehicle resulted in the deposition of a larger percentage of the total dose delivered in the nasal cavity, 76% on average, relative to delivery of the 0.5 ml frozen vehicle (Table 2). The majority of the remaining portion of the 0.2 ml dose was deposited in the nasopharynx (8%). Small percentages (< 9%) were observed on nasal wipes and in regions that, on planar images, overlay the esophagus and stomach. As seen for the 0.5 ml frozen vehicle, very small percentages of the signal (< 3%) were observed in regions that, on planar images, overlay the lungs and cranium, in patterns consistent with gamma ray scatter artifact from the esophagus/stomach and nasopharynx, respectively. Compared to the frozen vehicle formulation, the higher percentage of delivered dose of refrigerated vehicle being deposited in the nasal cavity is likely due to its reduced dose volume (0.2 ml vs. 0.5 ml).

Figure 4: Clearance of Frozen Vehicle and Refrigerated Vehicle from the Nasal Cavity (Study PPL-1014)

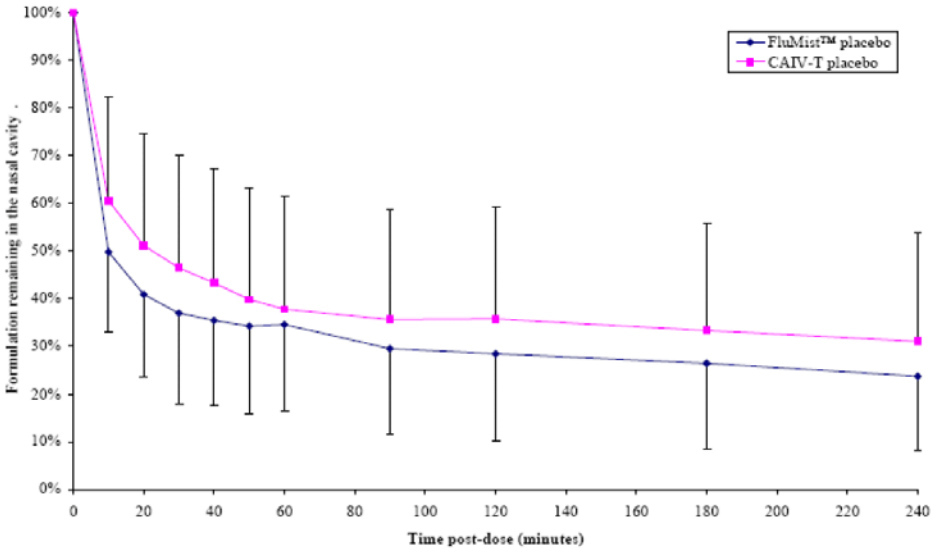


Table 2: Initial Deposition Pattern (Study PPL-1014)

Formulation	Region of Interest (mean +/- SD of % delivered dose)					
	Nasal cavity	Nasopharynx	Right and Left Lungs	Cranium (Brain)	Oesophagus and Stomach	Nasal Wipes
Frozen Vehicle	48.6 ± 13.2	14.2 ± 5.1	2.1 ± 1.1	2.5 ± 1.0	22.4 ± 14.7	10.2 ± 15.8
Refrigerated Vehicle	76.3 ± 18.5	7.8 ± 8.9	0.9 ± 0.9	2.5 ± 1.6	4.2 ± 6.5	8.3 ± 15.7

Source: Section 5.3.3.1.1, PPL-1014 CSR, Table 11.1

In summary, in vivo distribution studies in adults have shown that the majority of the dose of a radio-labelled refrigerated vaccine vehicle delivered by the same device that is used to deliver live, attenuated virus-containing vaccine was deposited in the nasal cavity with little or no measurable deposition in the lower airways and lungs, which is consistent with the relatively large droplet size of the spray material.

2.4.3. Pharmacodynamics

Pharmacodynamic of a vaccine relates to its interaction with the immune system.

Studies on immunogenicity include 2 clinical studies conducted by the Applicant to demonstrate the clinical comparability of the quadrivalent and trivalent formulations of live attenuated influenza vaccine in individuals 2 to 49 years of age. The comparable immune responses to Fluenz Tetra and FluMist demonstrated in these studies of Fluenz Tetra support the extrapolation to Fluenz Tetra of the extensive efficacy data for Fluenz/FluMist.

These studies are discussed in detail in the Clinical Efficacy section of this assessment report.

2.4.4. Conclusions on clinical pharmacology

Fluenz Tetra is formulated with a refrigerated vehicle and administered intranasally with the Becton Dickinson (BD) Accuspray™ delivery device. The same vehicle and the same delivery device have been tested in Study PPL-1014 which was previously submitted as part of the marketing

authorisation application for Fluenz. This study showed that the radio-labelled vehicle was mainly deposited in the nasal cavity of human adults with little or no measurable deposition in the lower airways and lungs. Hence, it is expected that Fluenz Tetra distribution pattern follows a similar fashion.

2.4.5. Clinical efficacy

As mentioned above, this application consists of a bridging strategy, previously agreed with EMA Scientific Advice, which aims at demonstrating that the immune responses generated by the quadrivalent vaccine are statistically non-inferior to the immune responses generated by the trivalent Fluenz vaccine. Based on immunogenicity equivalence of the quadrivalent and trivalent vaccines, the Applicant proposes that the efficacy data generated during the clinical development of Fluenz/FluMist be extrapolated to the quadrivalent vaccine Fluenz Tetra.

Also, in the dossier for Fluenz Tetra the Applicant included efficacy and related information from 43 clinical studies previously reviewed in the Fluenz MAA; the narrative for one of these studies (Study FM026) has been updated to include nasal IgA responses. Narratives for 2 studies (MI-CP114 and MI-CP128) were also provided as these studies were ongoing at the time of the Fluenz MAA submission. These data are considered overall supportive for the efficacy of Fluenz Tetra as well, however as they have been already assessed in the context of the Fluenz dossier, they are not described further in this report.

2.4.6. Dose response studies

No dose-response studies have been performed with Fluenz Tetra.

Each 0.2 ml dose of Fluenz Tetra contains $10^{7.0 \pm 0.5}$ FFU each of 4 ca, ts, att, 6:2 reassortant influenza strains (A/H1N1, A/H3N2, B/Victoria lineage, and B/Yamagata lineage). The proposed total potency specification for Fluenz Tetra is based on the maximum potential potency for the current trivalent vaccine. As in Fluenz/FluMist, each strain is present at $10^{7.0 \pm 0.5}$ FFU/0.2 ml dose; however, the maximum calculated total virus content of Fluenz Tetra is limited to 10^8 FFU per 0.2 ml dose, which is equivalent to the maximum calculated total virus content for Fluenz/FluMist (i.e., if each of the 3 strains in FluMist were present at $10^{7.5}$ FFU per dose). Vaccine manufactured according to these strain-specific virus potency specifications has been extensively studied in children and adults during the development of Fluenz/FluMist.

Two studies (D153-P513 and AV002/AV002-2) provide data regarding the dose-dependent immunogenicity of Fluenz/FluMist in paediatric and adult subjects. Study D153-P513 also showed that a 2-dose regimen of FluMist at a dosage level of 10^7 FFU per strain was statistically significantly more protective against culture-confirmed influenza illness in subjects 6 to < 36 months of age than a 2-dose regimen at a dosage level of 10^6 FFU or 10^5 FFU per strain.

Two additional studies (AV006 Year 1 and D153-P504) provide data regarding the efficacy of 1 dose vs. 2 doses of FluMist in paediatric subjects. In subjects 15 to 71 and 6 to 35 months of age, a single dose of FluMist was found to be efficacious against culture-confirmed influenza illness; estimates of efficacy were 88.8% and 57.7% respectively. However, the efficacy associated with a 2-dose primary series was numerically higher, and in most of the comparisons made within the studies, statistically higher than the efficacy of a single dose.

The proposed dosing recommendations for Fluenz Tetra in children are the same as what are currently recommended for Fluenz, where the number of doses recommended depends on the child's previous vaccination history.

2.4.7. Main studies

The clinical development programme for Q/LAIV consisted of three clinical immunogenicity studies: one pivotal study conducted in children (MI-CP208) and two supportive studies conducted in adults (MI-CP185 and MI-CP206), one of which used a different delivery system (MI-CP206). The immunogenicity of the Q/LAIV was compared with two Fluenz vaccines, each containing one of the B lineages included in the Q/LAIV. The paediatric studies with Fluenz provide supportive efficacy data. Study design is in accordance with recommendations in the "Guideline on Clinical Evaluation of New vaccines" (EMEA/CHMP/VWP/164653/05).

Pivotal Study MI-CP208

Methods

Study participants

The subjects in this study were male and female individuals 2 to 17 years of age. Subjects were divided into two groups by age: 9 to 17 years of age (one-dose group) and 2 to 8 years of age (two-dose group). The study population reflects the current pediatric age indication for FluMist marketed in the USA.

This randomized, double-blind, active controlled, multicenter study was designed to enroll approximately 500 subjects 9 to 17 years of age (one-dose group) and approximately 1,800 subjects 2 to 8 years of age (two-dose group) for a total sample size of approximately 2,300 subjects. Subjects were randomized in a 3:1:1 ratio to receive:

- Fluenz Tetra (quadrivalent live, attenuated influenza vaccine containing two type B influenza strains) (N = 1,380), or
- Trivalent FluMist containing an influenza B strain from the Yamagata lineage (FluMist-Y) (N = 460), or
- Trivalent FluMist containing an influenza B strain from the Victoria lineage (FluMist-V) (N = 460), with each of the FluMist influenza B strains matching one of the two B strains contained in Fluenz Tetra.

Randomization was stratified by age (2 to 8 years, 9 to 17 years). For subjects 2 to 8 years of age only, randomization was also stratified by history of previous seasonal influenza vaccination. Subjects were screened for the study within 30 days prior to randomization

Treatments

Test Product Dose, Mode of Administration, and Batch Number:

Investigational Product: Fluenz Tetra

Dose and Form: Each dose contained $10^{7.0 \pm 0.5}$ fluorescent focus units (FFU) of each of 4 cold-adapted (*ca*), attenuated (*att*), temperature sensitive (*ts*), 6:2 reassortant influenza strains (A/H1N1 [A/South Dakota/6/2007], A/H3N2 [A/Uruguay/716/2007], B of Victoria lineage [B/Malaysia/2506/2004], and B of Yamagata lineage [B/Florida/4/2006]).

Mode of Administration: Intranasal spray using the Becton Dickinson (BD) Accuspray™ device.

Batch/Lot Number: 0141700024

Reference Therapy, Dose, Mode of Administration, and Batch Number:

Investigational Product: FluMist containing a B strain of the Yamagata lineage (FluMist-Y)

Dose and Form: Each dose contained $10^{7.0 \pm 0.5}$ FFU of each of 3 *ca*, *ts*, *att*, 6:2 reassortant influenza strains (A/H1N1 [A/South Dakota/6/2007], A/H3N2 [A/Uruguay/716/2007], and B of Yamagata lineage [B/Florida/4/2006]).

Mode of Administration: Intranasal spray using the BD Accuspray device.

Batch/Lot Number: 0141500588

Investigational Product: FluMist containing a B strain of the Victoria lineage (FluMist-V)

Dose and Form: Each dose contained $10^{7.0 \pm 0.5}$ FFU of each of 3 ca, att, ts, 6:2 reassortant influenza strains (A/H1N1 [A/South Dakota/6/2007], A/H3N2 [A/Uruguay/716/2007], and B of Victoria lineage [B/Malaysia/2506/2004]).

Mode of Administration: Intranasal spray using the BD Accuspray device.

Batch/Lot Number: 0141700025

Duration of Treatment:

Subjects received either a single dose (subjects 9 to 17 years of age) of investigational product on Day 0 or two doses (subjects 2 to 8 years of age) of investigational product on Days 0 and 28.

Objectives

The primary objective of this study was to demonstrate the immunologic non-inferiority of Fluenz Tetra to FluMist in children 2 to 17 years of age by comparing the post dose strain-specific geometric mean titres (GMTs) of serum hemagglutination inhibition (HAI) antibody.

The secondary objectives of this study were:

1. To estimate the proportion of subjects 2 to 17 years of age who experienced post dose strain-specific HAI antibody seroresponse;
2. To estimate the proportion of subjects 2 to 17 years of age who achieved a post dose strain-specific HAI antibody titre ≥ 32;
3. To assess the safety and tolerability of two doses of Fluenz Tetra in subjects 2 to 8 years of age and of a single dose of Fluenz Tetra in subjects 9 to 17 years of age.

Outcomes/endpoints

The primary endpoint was the post dose strain-specific serum HAI antibody GMT, regardless of baseline serostatus. Immunologic non-inferiority of Fluenz Tetra to FluMist was demonstrated if the post dose strain-specific serum HAI antibody GMTs in the Fluenz Tetra arm were non-inferior to those in the FluMist arms for all 4 strains.

The post dose serum HAI antibody GMTs for A/H1N1 and A/H3N2 strain in Fluenz Tetra were compared to those in the combined FluMist-Y and FluMist-V arms, and the post dose serum HAI antibody GMTs for the B strains of Yamagata and Victoria lineage in Fluenz Tetra were compared to those in the FluMist-Y arm and FluMist-V arm, respectively. The non-inferior immune response was assessed by evaluating the upper bound of the two-sided 95% confidence intervals (CIs) for the strain-specific HAI antibody GMT ratios (FluMist divided by Fluenz Tetra) to the non-inferiority margin of 1.5. If the upper bounds of 95% CIs were ≤ 1.5 for all 4 strains, the immunologic non-inferiority of Fluenz Tetra compared to FluMist was declared.

No multiplicity adjustment was planned or implemented for the primary endpoint. Each of the 4 strain-specific non-inferiority comparisons carried a one-sided 2.5% type one error rate; therefore the overall type one error rate associated with simultaneous coverage by all 4 CIs was necessarily no more than 2.5%.

The analyses of the primary endpoint were based on the Immunogenicity Population. For all analyses of immune responses, a value of 2 was imputed for titres reported as < 4. Geometric mean titres for the strain-specific influenza antibody measurements were defined as:

$GMT = antilog_y (mean [log_y x])$

where x was the strain-specific HAI antibody titre and y was the natural logarithm.

The statistical hypothesis testing for the primary endpoint for Fluenz Tetra was:

H_0: $R_j > 1.5$, for any j

H_A: $R_j \leq 1.5$, for all j

Where R_j was any of the 4 strain-specific post immunogenicity dose GMT ratios:

- (FluMist-Y) / (Fluenz Tetra) for B/Yamagata strain
- (FluMist-V) / (Fluenz Tetra) for B/Victoria strain
- (FluMist-Y + FluMist-V) / (Fluenz Tetra) for A/H1N1 strain
- (FluMist-Y + FluMist-V) / (Fluenz Tetra) for A/H3N2 strain

Sample size

For the primary immunogenicity endpoint, with a sample size of 1,380 subjects in the Fluenz Tetra arm and 460 subjects in each of the two FluMist arms and the non-inferiority margin of 1.5 for GMT ratios, this study provided at least 92% power to demonstrate the immunologic non-inferiority of Fluenz Tetra compared to FluMist measured by the post immunogenicity dose serum HAI antibody GMT ratios (FluMist divided by Fluenz Tetra) for all of the 4 strains simultaneously if the true GMT ratio was ≤ 1.1 for all 4 strains.

Randomisation

Eligible subjects were randomized in a 3:1:1 ratio to receive Fluenz Tetra, trivalent FluMist containing an influenza B strain from the Yamagata lineage (FluMist-Y), or trivalent FluMist containing an influenza B strain from the Victoria lineage (FluMist-V). An interactive voice response system (IVRS) system was used at screening (all subjects screened), at randomization/assignment of Dose 1 (all eligible subjects), and assignment of Dose 2 (for randomized subjects age 2 to 8 years of age).

The IVRS that was used to assign the SID number to each subject at screening was used again for randomizing each eligible subject to a treatment arm and assigning investigational product kit number(s) and BD Accuspray device number(s).

The randomization incorporated a block design and stratification by age (2 to 8 years, 9 to 17 years). For subjects 2 to 8 years of age only, randomization was also stratified by previous seasonal influenza vaccination history.

Blinding (masking)

This was a double-blind study; therefore, neither the subject/legal representative nor any of the investigator or sponsor staff who were involved in the treatment or clinical evaluation of the subjects was aware of the treatment received.

Two formal analyses were planned for the study. An interim analysis of the final immunogenicity and Day 28 post last dose safety assessments was submitted on 05 January 2011. Study investigators and other site staff, study subjects, and contract research organization (CRO) personnel (including site monitors) directly associated with the conduct of this study remained blinded to the treatment assignment for individual subjects until the completion of the study. The detailed plan was documented in a separate unblinding memo prior to the unblinding. This final clinical study report (CSR) includes the previously reported final immunogenicity data and updates the safety data to provide final safety data collected through 180 days post last dose.

Statistical methods

Intent-to-treat Population

The Intent-to-Treat (ITT) Population includes all randomized subjects. Treatment arm will be assigned according to the initial randomization regardless of whether subjects received any investigational product or received an investigational product different from that to which they were randomized.

Safety Population

The Safety Population includes all subjects who received any investigational product and had any safety follow-up. Treatment arm for safety analysis will be assigned according to the actual treatment received at Dose I.

Immunogenicity Population

The Immunogenicity Population includes all subjects who received investigational product and had post dose HAI antibody measurement as defined below and had no protocol deviation judged to have the potential to interfere with the generation or interpretation of an immune response. Treatment arm for immunogenicity analysis will be assigned according to the actual treatment received at Dose 1.

- One-Dose group (9 to 17 years of age): received a dose of study vaccine and had post dose HAI antibody measurement;
- Two-Dose group (2 to 8 years of age) with history of prior seasonal influenza vaccination: received a dose of study vaccine at Dose 1 and had post dose 1 HAI antibody measurement;
- Two-Dose group (2 to 8 years of age) without history of prior seasonal influenza vaccination: received a dose of study vaccine at Doses 1 and 2 and had post Dose 2 HAI antibody measurement.

Primary endpoint

Strain-specific GMTs will be summarized by treatment group and by sample time (baseline or post dose strain-specific HAI). Corresponding two-sided 95% confidence intervals (95% CIs) for the GMTs and the ratios of post dose GMTs for the specified comparisons will be constructed using a percentile-based bootstrap method. In addition, for the calculation of 95% CIs for GMTs and GMT ratios in the overall population, the percentile-based bootstrap method will be stratified by baseline titre (titre≤ 4 and titre > 4), Specifically, ten thousand bootstrap data sets will be constructed by drawing replicates with replacement from each of the 4 cells (two treatment groups and two baseline titre sets; it could be 5 or 6 cells if there are missing baseline titres) of observed data. The number of replicates drawn from each cell in this fashion will be equal to the observed sample size within each cell. For each of the 10,000 bootstrap data sets, the overall GMT or GMT ratio will be calculated. The 95% CI will be constructed based upon the 2.5^{th} and 97.5^{th} percentiles of the 10,000 GMTs or GMT ratios.

The post dose serum HAI antibody GMTs for the A/H1N1 and A/H3N2 strains in Fluenz Tetra will be compared to those in the combined FluMist-Y and FluMist-V arms, and the post dose serum HAI

antibody GMTs for the B strains of Yamagata and Victoria lineage in Fluenz Tetra will be compared to those in the FluMist-Y arm and FluMist-V arm, respectively. If the upper bound of the 2-sided 95% CIs for the strain-specific HAI antibody GMT ratios (FluMist divided by Q/LAIV) is ≤ 1.5 for all 4 strains, the immunologic non-inferiority of Fluenz Tetra compared to FluMist will be declared. No multiplicity adjustment is planned for the primary endpoint.

Results

The study primary objective of demonstrating immunologic non-inferiority of Q/LAIV to two formulations of Fluenz in subjects 2 to 17 years of age by comparing the 4 strain-specific GMTs by post vaccination HAI assay was achieved. An analysis of GMFR in antibody titres measured by HAI supports the primary endpoint conclusion. In general, secondary endpoints supported the conclusion that the immune responses to Q/LAIV and Fluenz were similar, with two exceptions:

- vaccine immunogenicity was greater in the Fluenz group than in the Q/LAIV arm for the rate of seroconversion/seroresponse to A/H1N1 in the serosusceptible subgroup;
- the proportion of subjects achieving an HAI antibody titre ≥ 32 to the B/Yamagata strain in all subjects and based on baseline serostatus was greater for Fluenz than for Q/LAIV.

Based on the primary endpoint and supported by the secondary endpoint and by the post hoc analyses, the immune response to Q/LAIV can be considered as not inferior to the immune response to Fluenz. However, the relevance of the non-inferiority analysis is questioned in the case of A/H1N1 and A/H3N2 where a very low or zero response to vaccination was observed.

In general the results from NAI and neutralisation show the same trend as HAI: a good response to the B-strains in seronegative subjects and low or negligible response to H1N1 and H3N2. The exception is the good response against H1N1 when measured by microneutralisation (rate of seroconversion = 44-59%).

Summary of the main study

The following tables summarise the results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment.

Table 3: Summary of Results for trial MI-CP208

Title: A Randomized, Double-Blind, Active Controlled Study to Evaluate the Immunogenicity of Quadrivalent LAIV in Children		
Study identifier	**MI-CP208**	
Design	Randomized, double-blind, active-controlled, 3-arm, parallel-group	
	Duration of main phase:	One-dose Group : 180 to 187 days post Dose 1 (Day 0) Two-dose Group: 180 to 187 days post Dose 2 (28 to 35 days after post Dose 1)
	Duration of Run-in phase:	Screening between Day-30 and Day 0
	Duration of Extension phase:	Not applicable
Hypothesis	Non-inferiority Immunologic non-inferiority of Q/LAIV to Fluenz is considered to have been demonstrated if the post dose strain-specific serum Hemagglutination Inhibition (HAI) antibody geometric mean titres (GMTs) in the Q/LAIV arm were non-inferior to those in the Fluenz arms for all 4 strains.	

Treatments groups	Q/LAIV		Quadrivalent live attenuated influenza vaccine, 1385 patients randomized
	Fluenz-Y		Trivalent Fluenz containing influenza B strain from the Yamagata lineage, 464 patients randomized
	Fluenz-V		Trivalent Fluenz containing influenza B strain from the Victoria lineage, 463 patients randomized
Endpoints and definitions	Primary endpoint	GMT	The post dose strain-specific serum HAI antibody GMT, regardless of baseline serostatus.
	Secondary endpoints	Seroresponse (%)	The proportion of subjects who experienced post dose strain-specific HAI antibody seroresponse by baseline serostatus. Seroresponse defined as a ≥ 4-fold rise in HAI titre from baseline.
		HAI≥32 (%)	The proportion of subjects who achieved a post dose strain-specific HAI antibody titre ≥ 32 by baseline serostatus
	Other endpoints	GMFR	Geometric mean fold rise from baseline in HAI titres
Database lock	Not reported in the CSR		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Immunogenicity Population: All subjects who received a full dose of investigational product, had post dose HAI antibody measurements, and had no protocol deviation judged to have the potential to interfere with the generation or interpretation of an immune response. Dose groups: • One-dose group (9 to 17 years of age): received a dose of investigational product and had post dose HAI antibody measurement; • Two-dose group (2 to 8 years of age) with history of prior seasonal influenza vaccination: received a dose of investigational product at Dose 1 and had post Dose 1 HAI antibody measurement; • Two-dose group (2 to 8 years of age) without history of prior seasonal influenza vaccination: received a dose of investigational product at Doses 1 and 2 and had post Dose 2 HAI antibody measurement. Immunogenicity time point: ▪ 28 to 25 days after Dose 1 (for all subjects aged 9 to 17 years of age and subjects 2 to 8 years of age with a history of prior seasonal influenza vaccination) ▪ 28 to 35 days after Dose 2 for subjects 2 to 8 years of age with no history of prior seasonal influenza vaccination.		
Primary endpoint	Ratio of Post Immunogenicity Dose GMTs of HAI Antibody		
Descriptive statistics and estimate variability	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1327	883
	GMT	16.7	17.9

	95% CI	(15.9, 17.6)	(16.8, 19.1)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1327	883
	GMT	27.7	28.8
	95% CI	(26.1, 29.4)	(26.7, 31.1)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1327	445
	GMT	49.6	59.8
	95% CI	(46.6, 52.8)	(53.7, 66.7)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1327	437
	GMT	35.4	37.0
	95% CI	(33.3, 37.7)	(33.4, 41.0)

Effect estimate per comparison	Primary endpoint (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMT Ratio	1.07
		95% CI	(0.98, 1.16)
	Primary endpoint (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMT Ratio	1.04
		95% CI	(0.94, 1.14)
	Primary endpoint (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y**
		GMT Ratio	1.21
		95% CI	(1.07, 1.37)
	Primary endpoint (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V**
		GMT Ratio	1.05
		95% CI	(0.93, 1.18)
Notes	The All Fluenz group refers to data from both the Fluenz-Y arm and the Fluenz-V arm combined. The immune response of Q/LAIV was declared non-inferior to that of trivalent Fluenz if the upper bound for each of the four 95% CIs for post-dose strain-specific GMT ratios was ≤ 1.5.		

Analysis description	**Supportive Analysis**	
Analysis population and time point description	Immunogenicity Population Immunogenicity time point	
Endpoint	GMFR in HAI antibody titres	
Descriptive statistics		

	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1320	878
	GMFR	1.13	1.21
	95% CI	(1.10, 1.17)	(1.16, 1.27)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1321	879
	GMFR	1.06	1.05
	95% CI	(1.03, 1.09)	(1.00, 1.09)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1321	441
	GMFR	3.57	4.05
	95% CI	(3.37, 3.78)	(3.65, 4.49)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1321	437
	GMFR	2.77	2.73
	95% CI	(2.62, 2.93)	(2.49, 3.00)

Effect estimate per comparison	GMFR (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMFR Ratio	1.07
		95% CI	(1.01, 1.13)
	GMFR (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMFR Ratio	0.99
		95% CI	(0.94, 1.04)
	GMFR (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y**
		GMFR Ratio	1.13
		95% CI	(1.01, 1.27)
	GMFR (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V**
		GMFR Ratio	0.99
		95% CI	(0.88, 1.10)
Notes	A value of 2 was assigned for an HAI titre reported as < 4. Ratio = GMFR in comparator/GMFR in Q/LAIV. Confidence intervals based on bootstrapping method.		
Analysis description	**Secondary analysis**		

Analysis population and time point description	Immunogenicity Population. Subjects must also have had a baseline HAI measurement to be included in the analysis set. Immunogenicity time point.		
Secondary Endpoint	Proportion of subjects who experienced post dose strain-specific HAI antibody seroresponse by baseline serostatus.		
Descriptive statistics and estimate variability	Baseline Serostatus	**Seronegative**	
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	460	321
	Seroresponse (%)	14.6	19.6
	95% CI	(11.5, 18.1)	(15.4, 24.4)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	364	244
	Seroresponse (%)	9.9	11.1
	95% CI	(7.0, 13.4)	(7.4, 15.7)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	483	165
	Seroresponse (%)	83.0	84.8
	95% CI	(79.4, 86.3)	(78.5, 89.9)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	487	159
	Seroresponse (%)	68.8	73.6
	95% CI	(64.5, 72.9)	(66.0, 80.3)
Effect estimate per comparison	Seroresponse (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-5.1
		95% CI	(-10.6, 0.2)
	Seroresponse (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-1.2
		95% CI	(-6.5, 3.7)
	Seroresponse (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-1.8
		95% CI	(-7.8, 5.1)
	Seroresponse (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	-4.8

		95% CI	(-12.4, 3.6)
Descriptive statistics and estimate variability	Baseline Serostatus	**Serosusceptible**	
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	569	392
	Seroresponse (%)	12.7	17.6
	95% CI	(10.1, 15.7)	(14.0, 21.7)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	435	298
	Seroresponse (%)	9.9	9.4
	95% CI	(7.2, 13.1)	(6.3, 13.3)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	588	192
	Seroresponse (%)	79.1	81.3
	95% CI	(75.6, 82.3)	(75.0, 86.5)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	620	191
	Seroresponse (%)	66.1	69.6
	95% CI	(62.3, 69.9)	(62.6, 76.1)
Effect estimate per comparison	Seroresponse (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-4.9
		95% CI	(-9.7, -0.4)
	Seroresponse (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	0.5
		95% CI	(-4.1, 4.8)
	Seroresponse (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-2.2
		95% CI	(-8.2, 4.7)
	Seroresponse (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	-3.5
		95% CI	(-10.7, 4.3)
Descriptive statistics and estimate variability	Baseline Serostatus	**All**	
	Strain	**A/H1N1**	

	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1320	878
	Seroresponse (%)	6.3	8.2
	95% CI	(5.0, 7.7)	(6.5, 10.2)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1321	879
	Seroresponse (%)	3.9	3.6
	95% CI	(3.0, 5.1)	(2.5, 5.1)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1321	441
	Seroresponse (%)	43.4	44.9
	95% CI	(40.7, 46.1)	(40.2, 49.7)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1321	437
	Seroresponse (%)	39.1	38.4
	95% CI	(36.4, 41.8)	(33.9, 43.2)

Effect estimate per comparison	Seroresponse (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-1.9
		95% CI	(-4.2, 0.3)
	Seroresponse (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	0.3
		95% CI	(-1.4, 1.9)
	Seroresponse (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-1.5
		95% CI	(-6.9, 3.8)
	Seroresponse (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	0.6
		95% CI	(-4.7, 5.8)
Notes	Strain-specific baseline serostatus was defined as follows: ▪ seronegative if baseline HAI antibody titres were ≤ 4 ▪ serosusceptible if baseline HAI antibody titres were ≤ 8. Two-sided exact CI for percentage. Rate difference: Q/LAIV rate – comparator rate. Two-sided based on standardized (score) statistic.		
Analysis description	**Secondary analysis**		

Analysis population and time point description	Immunogenicity Population Immunogenicity time point		
Secondary Endpoint	Proportion of subjects who achieved a post dose strain-specific HAI antibody titre ≥ 32 by baseline serostatus.		
Descriptive statistics and estimate variability	Baseline Serostatus	**Seronegative**	
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	460	321
	HAI≥32 (%)	5.2	5.6
	95% CI	(3.4, 7.7)	(3.4, 8.7)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	364	244
	HAI≥32 (%)	3.8	4.9
	95% CI	(2.1, 6.4)	(2.6, 8.4)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	483	165
	HAI≥32 (%)	60.9	70.9
	95% CI	(56.4, 65.2)	(63.3, 77.7)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	487	159
	HAI≥32 (%)	37.0	42.8
	95% CI	(32.7, 41.4)	(35.0, 50.8)
Effect estimate per comparison	HAI≥32 (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-0.4
		95% CI	(-3.9, 2.8)
	HAI≥32 (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-1.1
		95% CI	(-4.9, 2.2)
	HAI≥32 (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-10.0
		95% CI	(-17.9, -1.6)
	HAI≥32 (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	-5.8
		95% CI	(-14.7, 2.8)

Descriptive statistics and estimate variability	Baseline Serostatus		**Serosusceptible**	
	Strain		**A/H1N1**	
	Treatment group		**Q/LAIV**	**All Fluenz group**
	Number of subjects		569	392
	HAI≥32 (%)		5.1	6.1
	95% CI		(3.4, 7.2)	(4.0, 9.0)
	Strain		**A/H3N2**	
	Treatment group		**Q/LAIV**	**All Fluenz group**
	Number of subjects		435	298
	HAI≥32 (%)		4.8	4.4
	95% CI		(3.0, 7.3)	(2.3, 7.3)
	Strain		**B/Yamagata**	
	Treatment group		**Q/LAIV**	**Fluenz-Y**
	Number of subjects		588	192
	HAI≥32 (%)		60.9	69.3
	95% CI		(56.8, 64.9)	(62.2, 75.7)
	Strain		**B/Victoria**	
	Treatment group		**Q/LAIV**	**Fluenz-V**
	Number of subjects		620	209
	HAI≥32 (%)		41.1	17.7
	95% CI		(37.2, 45.1)	(12.8, 23.6)
Effect estimate per comparison	HAI≥32 (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**	
		Rate difference	-1.0	
		95% CI	(-4.2, 1.9)	
	HAI≥32 (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**	
		Rate difference	0.5	
		95% CI	(-2.9, 3.5)	
	HAI≥32 (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**	
		Rate difference	-8.4	
		95% CI	(-15.7, -0.5)	
	HAI≥32 (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**	
		Rate difference	-2.9	
		95% CI	(-10.9, 5.1)	
Descriptive statistics and estimate variability	Baseline Serostatus		**All**	
	Strain		**A/H1N1**	
	Treatment group		**Q/LAIV**	**All Fluenz group**

	Number of subjects	1327	883
	HAI≥32 (%)	43.1	43.8
	95% CI	(40.4, 45.8)	(40.5, 47.2)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1327	883
	HAI≥32 (%)	55.7	55.4
	95% CI	(53.0, 58.4)	(52.0, 58.7)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1327	445
	HAI≥32 (%)	76.5	81.6
	95% CI	(74.1, 78.7)	(77.7, 85.1)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1327	437
	HAI≥32 (%)	65.6	66.6
	95% CI	(63.0, 68.2)	(62.0, 71.0)

Effect estimate per comparison	HAI≥32 (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-0.7
		95% CI	(-4.9, 3.5)
	HAI≥32 (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	0.3
		95% CI	(-3.9, 4.5)
	HAI≥32 (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-5.1
		95% CI	(-9.2, -0.6)
	HAI≥32 (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	-1.0
		95% CI	(-6.0, 4.2)
Notes	Strain-specific baseline serostatus was defined as follows: ▪ seronegative if baseline HAI antibody titres were ≤ 4 ▪ serosusceptible if baseline HAI antibody titres were ≤ 8. Two-sided exact CI for percentage. Rate difference: Q/LAIV rate – comparator rate. Two-sided CI for rate difference based on standardized (score) statistic.		

Analysis description	**Post Hoc Analyses**
Analysis population	Immunogenicity Subset for NAI antibody analysis

Number of subjects	A/H1N1		A/H3N2		B/Yamagata		B/Victoria	
	Q/LAIV	**All Fluenz**	**Q/LAIV**	**All Fluenz**	**Q/LAIV**	**Fluenz-Y**	**Q/LAIV**	**Fluenz-V**
	555	355	561	359	539	178	560	177
Effect estimate per comparison	GMT Ratio of NAI Antibody (A/H1N1)		Comparison groups		**Q/LAIV, All Fluenz Group**			
			GMT Ratio		1.16			
			95% CI		(1.01, 1.35)			
	GMT Ratio of NAI Antibody (A/H3N2)		Comparison groups		**Q/LAIV, All Fluenz Group**			
			GMT Ratio		1.00			
			95% CI		(0.89, 1.12)			
	GMT Ratio of NAI Antibody (B/Yamagata)		Comparison groups		**Q/LAIV, Fluenz-Y**			
			GMT Ratio		1.03			
			95% CI		(0.86, 1.22)			
	GMT Ratio of NAI Antibody (B/Victoria)		Comparison groups		**Q/LAIV, Fluenz-V**			
			GMT Ratio		0.82			
			95% CI		(0.70, 0.96)			
Notes	Ratio = GMT in comparator divided by GMT in Q/LAIV. A value of 5 was assigned for NAI antibody titres reported as < 10. Confidence intervals based on bootstrapping method.							
Analysis population	Immunogenicity Subset for Microneutralization analysis							
Number of subjects	A/H1N1		A/H3N2		B/Yamagata		B/Victoria	
	Q/LAIV	**All Fluenz**	**Q/LAIV**	**All Fluenz**	**Q/LAIV**	**Fluenz-Y**	**Q/LAIV**	**Fluenz-V**
	259	173	239	156	273	101	267	82
Effect estimate per comparison	GMT Ratio of Microneutralization Antibody (A/H1N1)		Comparison groups		**Q/LAIV, All Fluenz Group**			
			GMT Ratio		0.86			
			95% CI		(0.66, 1.11)			
	GMT Ratio of Microneutralization Antibody (A/H3N2)		Comparison groups		**Q/LAIV, All Fluenz Group**			
			GMT Ratio		0.93			
			95% CI		(0.69, 1.26)			
	GMT Ratio of Microneutralization Antibody (B/Yamagata)		Comparison groups		**Q/LAIV, Fluenz-Y**			
			GMT Ratio		1.28			
			95% CI		(1.00, 1.62)			
	GMT Ratio of Microneutralization Antibody (B/Victoria)		Comparison groups		**Q/LAIV, Fluenz-V**			
			GMT Ratio		1.16			
			95% CI		(0.89, 1.51)			
Notes	Ratio = GMT in comparator divided by GMT in Q/LAIV. A value of 5 was assigned for MN microneutralization titres reported as < 10. Confidence intervals based on bootstrapping method.							

Analysis description		Post-hoc analyses					
		Post Immunogenicity Dose Strain-specific Seroconversion/Seroresponse Rates (Greater Than or Equal to 4-fold Rise in NAI Antibody Titer From Baseline) by Baseline Serostatus, Immunogenicity Population for Analysis of NAI Antibody (Q/LAIV Pediatric Study MI-CP208)					
Baseline Serostatus	**Strain**	**Q/LAIV n/N (%) [95% CI]**	**All FluMist n/N (%) [95% CI]**	**FluMist-Y n/N (%) [95% CI]**	**FluMist-V n/N (%) [95% CI]**	**Rate Difference**	
						Comparator	**Percentage Points**
All	A/H1N1	43/544 (7.9) [5.8, 10.5]	50/351 (14.2) [10.8, 18.3]	29/179 (16.2) [11.1, 22.4]	21/172 (12.2) [7.7, 18.1]	All FluMist	-6.3
	A/H3N2	9/556 (1.6) [0.7, 3.1]	8/357 (2.2) [1.0, 4.4]	6/181 (3.3) [1.2, 7.1]	2/176 (1.1) [0.1, 4.0]	All FluMist	-0.6
	B/Yamagata	279/532 (52.4) [48.1, 56.8]	--	92/177 (52.0) [44.4, 59.5]	64/171 (37.4) [30.2, 45.1]	FluMist-Y	0.5
	B/Victoria	309/553 (55.9) [51.6, 60.1]	--	94/182 (51.6) [44.1, 59.1]	75/175 (42.9) [35.4, 50.5]	FluMist-V	*13.0*
Seronegative	A/H1N1	33/312 (10.6) [7.4, 14.5]	41/195 (21.0) [15.5, 27.4]	23/97 (23.7) [15.7, 33.4]	18/98 (18.4) [11.3, 27.5]	All FluMist	-10.4
	A/H3N2	6/319 (1.9) [0.7, 4.0]	6/212 (2.8) [1.0, 6.1]	4/112 (3.6) [1.0, 8.9]	2/100 (2.0) [0.2, 7.0]	All FluMist	-0.9
	B/Yamagata	205/249 (82.3) [77.0, 86.9]	--	71/84 (84.5) [75.0, 91.5]	43/70 (61.4) [49.0, 72.8]	FluMist-Y	-2.2
	B/Victoria	205/217 (94.5) [90.5, 97.1]	--	65/76 (85.5) [75.6, 92.5]	55/65 (84.6) [73.5, 92.4]	FluMist-V	9.9
Seropositive	A/H1N1	10/232 (4.3) [2.1, 7.8]	9/156 (5.8) [2.7, 10.7]	6/82 (7.3) [2.7, 15.2]	3/74 (4.1) [0.8, 11.4]	All FluMist	-1.5
	A/H3N2	3/237 (1.3) [0.3, 3.7]	2/145 (1.4) [0.2, 4.9]	2/69 (2.9) [0.4, 10.1]	0/76 (0.0) [0.0, 4.7]	All FluMist	-0.1
	B/Yamagata	74/283 (26.1) [21.1, 31.7]	--	21/93 (22.6) [14.6, 32.4]	21/101 (20.8) [13.4, 30.0]	FluMist-Y	3.6
	B/Victoria	104/336 (31.0) [26.0, 36.2]	--	29/106 (27.4) [19.1, 36.9]	20/110 (18.2) [11.5, 26.7]	FluMist-V	12.8

Analysis description		Post-hoc analyses				
		Post Immunogenicity Dose Strain-specific Seroconversion/Seroresponse Rates (Greater Than or Equal to 4-fold Rise in Microneutralization Antibody Titer From Baseline) by Baseline Serostatus, Immunogenicity Population for Analysis of Microneutralization Antibody (Q/LAIV Pediatric Study MI-CP208)				
Baseline Serostatus	**Strain**	**Q/LAIV n/N (%) [95% CI]**	**All FluMist n/N (%) [95% CI]**	**FluMist-Y n/N (%) [95% CI]**	**FluMist-V n/N (%) [95% CI]**	**Rate Difference**

						Comparator	Percentage Points
All	A/H1N1	32/240 (13.3) [9.3, 18.3]	18/167 (10.8) [6.5, 16.5]	13/88 (14.8) [8.1, 23.9]	5/79 (6.3) [2.1, 14.2]	All FluMist	2.6
	A/H3N2	15/235 (6.4) [3.6, 10.3]	8/154 (5.2) [2.3, 10.0]	5/83 (6.0) [2.0, 13.5]	3/71 (4.2) [0.9, 11.9]	All FluMist	1.2
	B/Yamagata	169/272 (62.1) [56.1, 67.9]	--	63/101 (62.4) [52.2, 71.8]	9/84 (10.7) [5.0, 19.4]	FluMist-Y	-0.2
	B/Victoria	101/260 (38.8) [32.9, 45.1]	--	22/98 (22.4) [14.6, 32.0]	36/80 (45.0) [33.8, 56.5]	FluMist-V	-6.2
Seronegative	A/H1N1	23/39 (59.0) [42.1, 74.4]	12/27 (44.4) [25.5, 64.7]	9/14 (64.3) [35.1, 87.2]	3/13 (23.1) [5.0, 53.8]	All FluMist	14.5
	A/H3N2	10/59 (16.9) [8.4, 29.0]	7/34 (20.6) [8.7, 37.9]	4/21 (19.0) [5.4, 41.9]	3/13 (23.1) [5.0, 53.8]	All FluMist	-3.6
	B/Yamagata	153/188 (81.4) [75.1, 86.7]	--	57/65 (87.7) [77.2, 94.5]	8/51 (15.7) [7.0, 28.6]	FluMist-Y	-6.3
	B/Victoria	83/155 (53.5) [45.4, 61.6]	--	18/69 (26.1) [16.3, 38.1]	29/46 (63.0) [47.5, 76.8]	FluMist-V	-9.5
Seropositive	A/H1N1	9/201 (4.5) [2.1, 8.3]	6/140 (4.3) [1.6, 9.1]	4/74 (5.4) [1.5, 13.3]	2/66 (3.0) [0.4, 10.5]	All FluMist	0.2
	A/H3N2	5/176 (2.8) [0.9, 6.5]	1/120 (0.8) [0.0, 4.6]	1/62 (1.6) [0.0, 8.7]	0/58 (0.0) [0.0, 6.2]	All FluMist	2.0
	B/Yamagata	16/ 84 (19.0) [11.3, 29.1]	--	6/36 (16.7) [6.4, 32.8]	1/33 (3.0) [0.1, 15.8]	FluMist-Y	2.4
	B/Victoria	18/105 (17.1) [10.5, 25.7]	--	4/29 (13.8) [3.9, 31.7]	7/34 (20.6) [8.7, 37.9]	FluMist-V	-3.4

Analysis performed across trials (pooled analyses and meta-analysis)

Despite the similarities in study design between the 2 Q/LAIV studies, the immunogenicity data from the studies were not pooled due to the different age of the subjects enrolled. It is known that younger children have higher immune responses to the vaccine, as measured by serum HAI responses, and that adults tend to have modest responses in comparison. On the other hand, differences between the two studies in terms of dosing regimen, prior vaccine exposure and baseline serostatus limit the validity of a direct comparison of results across studies. Therefore analyses across studies have not been performed and this was considered acceptable.

Clinical studies in special populations

No special paediatric populations have been investigated.

Supportive studies

Table 4: Summary of results for trial MI-CP185

Title: A Randomized, Double-Blind, Active Controlled Study to Evaluate the Immunogenicity of MEDI3250 in Adults 18 to 49 Years of Age			
Study identifier	**MI-CP185**		
Design	Randomized, Double-Blind, Active-Controlled		
	Duration of main phase:		180 days post dose administration of vaccine (Day 0): ▪ 14 days for solicited symptoms ▪ 28 days for AEs ▪ 180 days for SAEs and new onset chronic diseases (NOCDs).
	Duration of Screening period: Duration of Extension phase:		A screening period of up to 30 days was permitted. Not applicable
Hypothesis	Non-inferiority. Immunologic non-inferiority of Q/LAIV to Fluenz would be considered to have been demonstrated if the post dose strain-specific serum HAI antibody GMTs in the Q/LAIV arm were non-inferior to those in the Fluenz arms for all 4 strains.		
Treatments groups	**Q/LAIV**		Quadrivalent live attenuated influenza vaccine. N (randomized)=1200
	Fluenz-Y		Trivalent Fluenz containing influenza B strain from the Yamagata lineage. N (randomized)=299
	Fluenz-V		Trivalent Fluenz containing influenza B strain from the Victoria lineage. N (randomized)=301
Endpoints and definitions	Primary endpoint	GMT	The post dose strain-specific serum HAI antibody GMT in all 4 strains, regardless of baseline serostatus
	Secondary endpoints	Seroresponse (%)	The proportion of subjects who experienced post dose strain-specific HAI antibody seroresponse by baseline serostatus. Seroresponse defined as a ≥ 4-fold rise in HAI titre from baseline.
		HAI≥32 (%)	The proportion of subjects who achieved a post dose strain-specific HAI antibody titre ≥ 32 by baseline serostatus
Database lock	Not reported in the CSR		
Results and Analysis			
Analysis description	**Primary Analysis**		

Analysis population and time point description	Number of subjects: - randomized: 1800 subjects - dosed: 1798 subjects - Immunogenicity Population: 1770 subjects. Immunogenicity Population: All subjects who received a full dose of investigational product, had post dose HAI antibody measurement, and had no protocol deviation judged to have the potential to interfere with the generation or interpretation of an immune response. Subjects identified with deviations judged to have the potential to interfere with the generation or interpretation of an immune response were excluded from the Immunogenicity Population prior to unblinding. Subjects were included in the treatment arm corresponding to the treatment received even if it was different from the randomized treatment. Immunogenicity time point: during the interval of Day 28 to Day 35 post dose		
Primary endpoint	Post dose strain-specific HAI antibody GMTs		
Descriptive statistics	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1181	589
	GMT	5.9	6.5
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1181	589
	GMT	7.5	7.8
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1181	292
	GMT	51.2	56.4
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1181	297
	GMT	36.5	33.6
Effect estimate per comparison	Primary endpoint (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMT Ratio	1.09
		95% CI	(1.01, 1.18)
	Primary endpoint (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMT Ratio	1.05
		95% CI	(0.96, 1.14)
	Primary endpoint (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y**
		GMT Ratio	1.10
		95% CI	(0.97, 1.25)

	Primary endpoint (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V**
		GMT Ratio	0.92
		95% CI	(0.82, 1.03)
Notes	The All Fluenz group refers to data from both the Fluenz-Y arm and the Fluenz-V arm combined. The immune response of Q/LAIV was declared noninferior to that of trivalent Fluenz if the upper bound for each of the four 95% CIs for post-dose strain-specific GMT ratios was ≤ 1.5.		
Analysis description	**Secondary analysis**		
Analysis population and time point description	Immunogenicity Population. Subjects must also have had a baseline HAI measurement to be included in the analysis set. Immunogenicity time point.		
Secondary Endpoint	Proportion of subjects who experienced post dose strain-specific seroresponse post dose by baseline serostatus. Seroresponse: ≥4-fold increase in HAI antibody titre from baseline.		
Descriptive statistics and estimate variability	Baseline Serostatus	**Serosusceptible**	
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	889	429
	Seroresponse (%)	6.6	7.0
	95% CI	(5.1, 8.5)	(4.8, 9.8)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	807	393
	Seroresponse (%)	6.6	6.4
	95% CI	(5.0, 8.5)	(4.2, 9.2)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	197	43
	Seroresponse (%)	33.5	39.5
	95% CI	(27.0, 40.6)	(25.0, 55.6)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	250	66
	Seroresponse (%)	36.8	42.4
	95% CI	(30.8, 43.1)	(30.3, 55.2)
Effect estimate per comparison	Seroresponse (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-0.4
		95% CI	(-3.5, 2.4)

	Seroresponse (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	0.2
		95% CI	(-3.0, 3.0)
	Seroresponse (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-6.0
		95% CI	(-22.3, 9.0)
	Seroresponse (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	-5.6
		95% CI	(-19.0, 7.2)

Descriptive statistics and estimate variability	Baseline Serostatus	**Seropositive**	
	Strain	**A/H1N1**	**All Fluenz group**
	Number of subjects	291	160
	Seroresponse (%)	0.7	0.6
	95% CI	(0.1, 2.5)	(0.0, 3.4)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	373	196
	Seroresponse (%)	1.6	0.0
	95% CI	(0.6, 3.5)	(0.0, 1.9)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	983	249
	Seroresponse (%)	5.3	5.2
	95% CI	(4.0, 6.9)	(2.8, 8.8)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	930	231
	Seroresponse (%)	5.7	3.0
	95% CI	(4.3, 7.4)	(1.2, 6.1)

Effect estimate per comparison	Seroresponse (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	0.1
		95% CI	(-2.8, 1.9)
	Seroresponse (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	1.6
		95% CI	(-0.3, 3.5)
	Seroresponse (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	0.1

		95% CI	(-3.6, 2.8)
	Seroresponse (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	2.7
		95% CI	(-0.7, 5.0)

Descriptive statistics and estimate variability	Baseline Serostatus	**All**	
	Strain	**A/H1N1**	**All Fluenz group**
	Number of subjects	1180	589
	Seroresponse (%)	5.2	5.3
	95% CI	(4.0, 6.6)	(3.6, 7.4)
	Strain		
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1180	589
	Seroresponse (%)	5.0	4.2
	95% CI	(3.8, 6.4)	(2.8, 6.2)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1180	292
	Seroresponse (%)	10.0	10.3
	95% CI	(8.3, 11.9)	(7.0, 14.3)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1180	297
	Seroresponse (%)	12.3	11.8
	95% CI	(10.5, 14.3)	(8.3, 16.0)

Effect estimate per comparison	Seroresponse (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-0.1
		95% CI	(-2.5, 2.0)
	Seroresponse (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	0.8
		95% CI	(-1.5, 2.7)
	Seroresponse (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-0.3
		95% CI	(-4.6, 3.3)
	Seroresponse (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	0.5
		95% CI	(-4.0, 4.3)

Notes	Strain-specific baseline serostatus was defined as follows: ▪ serosusceptible if baseline strain specific HAI antibody titre ≤ 8 ▪ seropositive if baseline strain specific HAI antibody titre > 8. A value of 2 was assigned for an HAI titre reported as < 4. Two-sided exact CI for percentage. Rate difference: Q/LAIV rate – comparator rate. Two-sided based on standardized (score) statistic.			
Analysis description	**Secondary analysis**			
Analysis population and time point description	Immunogenicity Population. Immunogenicity time point.			
Secondary Endpoint	Percentage of subjects who achieved a strain-specific HAI antibody titre ≥ 32 post dose by baseline serostatus.			
Descriptive statistics and estimate variability	Baseline Serostatus	**Seropositive**		
	Strain	**A/H1N1**		
	Treatment group	**Q/LAIV**	**All Fluenz group**	
	Number of subjects	889	429	
	HAI≥32 (%)	1.9	1.2	
	95% CI	(1.1, 3.0)	(0.4, 2.7)	
	Strain	**A/H3N2**		
	Treatment group	**Q/LAIV**	**All Fluenz group**	
	Number of subjects	807	393	
	HAI≥32 (%)	2.2	2.8	
	95% CI	(1.3, 3.5)	(1.4, 5.0)	
	Strain	**B/Yamagata**		
	Treatment group	**Q/LAIV**	**Fluenz-Y**	
	Number of subjects	197	43	
	HAI≥32 (%)	22.3	25.6	
	95% CI	(16.7, 28.8)	(13.5, 41.2)	
	Strain	**B/Victoria**		
	Treatment group	**Q/LAIV**	**Fluenz-V**	
	Number of subjects	250	66	
	HAI≥32 (%)	22.8	24.2	
	95% CI	(17.7, 28.5)	(14.5, 36.4)	
Effect estimate per comparison	HAI≥32 (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**	
		Rate difference	0.7	
		95% CI	(-0.9, 2.1)	
	HAI≥32 (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**	
		Rate difference	-0.6	

		95% CI	(-2.9, 1.2)
	HAI≥32 (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-3.2
		95% CI	(-18.9, 9.3)
	HAI≥32 (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	-1.4
		95% CI	(-14.0, 9.0)

Descriptive statistics and estimate variability	Baseline Serostatus	**Seropositive**	
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	291	160
	HAI≥32 (%)	59.1	59.4
	95% CI	(53.2, 64.8)	(51.3, 67.1)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	373	196
	HAI≥32 (%)	62.2	62.2
	95% CI	(57.1, 67.1)	(55.1, 69.1)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	983	249
	HAI≥32 (%)	85.1	86.3
	95% CI	(82.8, 87.3)	(81.4, 90.4)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	930	231
	HAI≥32 (%)	76.8	75.8
	95% CI	(73.9, 79.5)	(69.7, 81.1)

Effect estimate per comparison	HAI≥32 (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-0.3
		95% CI	(-9.6, 9.3)
	HAI≥32 (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-0.0
		95% CI	(-8.3, 8.4)
	HAI≥32 (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-1.2

		95% CI	(-5.6, 4.0)
	HAI≥32 (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	1.0
		95% CI	(-4.8, 7.5)

Descriptive statistics and estimate variability	Baseline Serostatus	**All**	
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1181	589
	HAI≥32 (%)	16.0	17.0
	95% CI	(14.0, 18.2)	(14.0, 20.3)
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1181	589
	HAI≥32 (%)	21.2	22.6
	95% CI	(18.9, 23.6)	(19.3, 26.2)
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1181	292
	HAI≥32 (%)	74.6	77.4
	95% CI	(72.0, 77.1)	(72.2, 82.1)
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1181	292
	HAI≥32 (%)	65.3	51.4
	95% CI	(62.5, 68.0)	(45.5, 57.2)

Effect estimate per comparison	HAI≥32 (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-1.0
		95% CI	(-4.8, 2.6)
	HAI≥32 (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		Rate difference	-1.4
		95% CI	(-5.6, 2.6)
	HAI≥32 (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y for the B/Yamagata strain**
		Rate difference	-2.8
		95% CI	(-7.9, 2.9)
	HAI≥32 (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V for the B/Victoria strain**
		Rate difference	1.0

		95% CI	(-5.0, 7.2)
Notes	Strain-specific baseline serostatus was defined as follows: ▪ serosusceptible if baseline strain specific HAI antibody titre ≤ 8 ▪ seropositive if baseline strain specific HAI antibody titre > 8. A value of 2 was assigned for an HAI titre reported as < 4. Two-sided exact CI for percentage. Rate difference: Q/LAIV rate – comparator rate. Two-sided based on standardized (score) statistic.		

Analysis description	**Post Hoc Analysis**		
Analysis population and time point description	Immunogenicity Population Immunogenicity time point		
Endpoint	Geometric mean fold rise (GMFR) in HAI antibody titres		
Descriptive statistics			
	Strain	**A/H1N1**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1,180	589
	GMFR	1.16	1.14
	Strain	**A/H3N2**	
	Treatment group	**Q/LAIV**	**All Fluenz group**
	Number of subjects	1,180	589
	GMFR	1.13	1.12
	Strain	**B/Yamagata**	
	Treatment group	**Q/LAIV**	**Fluenz-Y**
	Number of subjects	1,180	292
	GMFR	1.37	1.27
	Strain	**B/Victoria**	
	Treatment group	**Q/LAIV**	**Fluenz-V**
	Number of subjects	1,180	297
	GMFR	1.40	1.35

Effect estimate per comparison	GMFR (A/H1N1)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMFR Ratio	0.98
		95% CI	(0.93, 1.04)
	GMFR (A/H3N2)	Comparison groups	**Q/LAIV, All Fluenz Group**
		GMFR Ratio	0.99
		95% CI	(0.94, 1.06)
	GMFR (B/Yamagata)	Comparison groups	**Q/LAIV, Fluenz-Y**
		GMFR Ratio	0.93
		95% CI	(0.85, 1.01)

	GMFR (B/Victoria)	Comparison groups	**Q/LAIV, Fluenz-V**
		GMFR Ratio	0.97
		95% CI	(0.89, 1.04)
Notes	A value of 2 was assigned for an HAI titre reported as < 4. Ratio = GMFR in comparator/GMFR in Q/LAIV. Confidence intervals based on bootstrapping method. This *post hoc* analysis was performed because of the differences between arms in baseline GMTs.		

Study MI-CP185 overall demonstrates immunologic non-inferiority between Q/LAIV and two formulations of Fluenz in subjects 18 to 49 years of age by comparing the 4 strain-specific GMTs of post-vaccination antibody titres measured by HAI. An analysis of GMFR in HAI antibody titres supports the primary endpoint conclusion. In general, secondary endpoint data were consistent with the primary endpoint. The results from the post hoc analyses also supported the primary endpoint conclusion.

Based on the primary endpoint with support from the secondary endpoint of seroconversion/seroresponse data, the immune response to Q/LAIV can be considered as not inferior to the immune response induced by Fluenz.

Summary of results for trial MI-CP206

This was a randomized, partially blind active-controlled study to evaluate the immunogenicity of MEDI8662 in adults 18 to 49 years of age.

The study was conducted at 18 sites in the United States of America from August 2009 to March 2010. The primary objective of this study was to demonstrate the immunologic non-inferiority of Fluenz Tetra administered intranasally through a different delivery system (Fluenz Tetra-BFS) to 2 trivalent formulations of licensed FluMist (delivered intranasally using the Accuspray delivery device) by comparing the strain-specific geometric mean titres (GMTs) post dosing.

The secondary objectives of this study were:

1. To estimate the proportion of subjects who experienced strain-specific hemagglutination inhibition (HAI) seroresponse following the dose of Fluenz Tetra-BFS, defined as a minimum 4-fold rise in post-vaccination HAI antibody titre, by baseline serostatus.
2. To estimate the proportion of subjects who achieved a strain-specific HAI titre ≥ 32 following the dose of Fluenz Tetra-BFS, by baseline serostatus.
3. To assess the safety and tolerability of Fluenz Tetra-BFS.
4. To determine the acceptability of the BFS dosing unit as a vaccine delivery system to vaccine recipients.

In summary, study MI-CP206 met its primary endpoint demonstrating the immunologic non-inferiority of Q/LAIV-BFS to two formulations of Fluenz by comparing the 4 strain-specific HAI antibody GMTs post dosing. The upper bound for each of the four 95% CIs for the post dose GMT ratios was ≤ 1.5 (ranging between 1.0 and 1.1).

Secondary endpoint data were consistent with the primary endpoint. For all subjects, regardless of baseline serostatus, the post dose seroconversion/seroresponse rates were low (≤ 10%) but similar in the Q/LAIV-BFS group and in the All Fluenz group. In all subjects, regardless of baseline serostatus, the percentage of subjects achieving a post dose HAI antibody titre ≥ 32 was similar between the Q/LAIV-BFS and all Fluenz groups.

2.4.8. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical development programme for Q/LAIV consisted of three studies: one pivotal study conducted in children (MI-CP208) and two supportive studies conducted in adults (MI-CP185 and MI-CP206) one of which used a different delivery system (MI-CP206). The efficacy of Q/LAIV was compared with two Fluenz vaccines, each containing one of the B lineages used in Q/LAIV. The paediatric Fluenz studies provide supportive efficacy data.

The studies were designed and conducted in accordance with the principles of the Declaration of Helsinki and in compliance with the principles of Good Clinical Practice. The study designs are in accordance with recommendations made in the EMA "Guideline on Clinical Evaluation of New vaccines". The number of paediatric subjects included in the Q/LAIV development programme is limited. Also for this reason the Applicant is asked to provide a post-authorisation study to evaluate the efficacy of Q/LAIV in children aged 24 months to less than 18 years.

All Q/LAIV studies were conducted in the United States exclusively. Demographic characteristics of the Q/LAIV paediatric study were similar to the Fluenz studies and overall the patient population is considered to be representative of the general paediatric population. However it was noted that children with co-morbidities, and especially immune-compromised children, were generally excluded from the studies and subjects of Asian and African descent were underrepresented. In both studies (MI-CP208 and MI-CP185), the majority of the patients were Caucasian: 71% and 76% in Studies MI-CP208 and MI-CP185, respectively. Approximately 20% of the patients were Black or African-American. The number of subjects in the 2 to 8 years age subset was more than 3 times higher than in the 9 to 17 years age subset (approx. 3,5:1). In the younger age group, the majority of children had previously received an influenza vaccine (2,5:1).

The relevance of measuring humoral immunity for a LAIV instead of mucosal immunity could be questioned. However, a standardized and validated method for isolation and quantitative analysis of mucosal antibodies is currently not available and such development would technically be very challenging (if at all possible). Moreover, no correlates of protection have been established.

Efficacy data and additional analyses

In accordance with the primary objective of study **MI-CP208** and with generally accepted parameters for the determination of non-inferior immunogenicity, immune responses to vaccination against each of the four virus strains contained in Q/LAIV were not inferior to immune responses induced against the same strains by the licensed (trivalent) Fluenz.

Overall, secondary endpoints support this conclusion, despite the rate of seroconversion/seroresponse to A/H1N1 in the sero-susceptible subgroup, which was statistically higher in the Fluenz group than in the Q/LAIV arm, and likewise the proportion of subjects achieving an HAI antibody titre ≥ 32 for the B/Yamagata strain in all subjects regardless of baseline serostatus. However based on the overall results, these differences were not considered to be of clinical relevance. The analysis of GMFR from baseline also supports the conclusion that responses to Q/LAIV and Fluenz are similar.

The non-inferiority analysis is relevant to investigate if the addition of a fourth virus strain affects negatively the immune responses induced by the vaccine as a surrogate for protection, despite the lack of an established correlate of protection. Lower immune responses against H1N1 and H3N2 were shown, which have been satisfactorily addressed by the Applicant based on the following observations:

- Different receptor binding specificity for A vs. B strains and among B-lineages can reduce risk of interference.

- Non-inferiority assessed by HAI geometric mean fold rise ratio (GMFR), which control for differences in baseline titres, shows that the ratio of Fluenz/FluMist to Fluenz Tetra is close to 1.0 in most cases.
- Non-inferiority assessed using neutralising antibody GMFR data in children 2 to 5 years shows that the ratio of Fluenz/FluMist to Fluenz Tetra is close to 1.0.
- The results from non-clinical development, including from 4 challenge studies where virus shedding was measured, show that Fluenz Tetra and Fluenz/FluMist equally block viral replication.
- A number of developmental clinical studies including a challenge study indicate that monovalent, bivalent and trivalent formulations induce similar level of protection (albeit different populations, strains and design make the comparison difficult).

These observations indicate that the theoretical risk of immune interference by adding a fourth strain to the vaccine is small and outweighed by the advantage of covering both B lineages at the same time. Even though some differences in favour of the trivalent Fluenz/FluMist were noticed (i.e. higher seroconversion rate in seronegative children aged 2 to < 4 years and a GMFR ratio of 1.45 for seronegative subjects against H1N1 after dose 2), the overall picture seems similar. Especially the neutralising data are reassuring, although the number of subjects analysed is not extensive. The satisfactory protection data shown in ferrets by the challenge studies comparing tetravalent with trivalent LAIVs are also considered indicative of lack of immune interference. Finally, the Applicant agrees to perform a post-authorisation effectiveness study as detailed in the RMP.

Concerning duration of immune response, in the pivotal paediatric study MI-CP208, strain-specific antibody levels were measured 4 weeks after the last dose. Previous data with Fluenz/FluMist show persistence of protection against disease and immunogenicity for up to 2 years post-vaccination.

The supportive study **MI-CP185** met its primary objective of demonstrating the immunologic non-inferiority of Q/LAIV vs. 2 formulations of Fluenz in subjects 18 to 49 years of age by comparing the 4 strain-specific HAI antibody GMTs post dosing. The immune response to Q/LAIV is considered non-inferior vs. Fluenz because the upper bound for each of the four 95% CIs for the GMT ratios (Fluenz divided by Q/LAIV) was ≤ 1.5.

The Applicant states that the data from 2 separate Fluenz studies in young subjects (D153-P501 and D153-P504) showed that receipt of Fluenz in Year 1 was associated with statistically significant efficacy against culture-positive influenza illness that persisted into the subsequent season, and that additional benefit was afforded by receipt of the second season revaccination. Data from another study in young subjects (AV006) showed that there was a high persistence of antibody against A/H3N2 and B strains (but not A/H1N1 strains) approximately 1 year after initial vaccination with a 1 or 2-dose primary regimen of Fluenz. The CHMP agrees that additional benefit from the second year revaccination is demonstrated, and in addition the effectiveness study planned for the post-authorisation phase should provide further data on consequent revaccination. The Applicant confirmed that the study will be sufficiently powered to detect if similar vaccine efficacy is maintained after Q/LAIV repeated use vs. subjects who received no influenza vaccine in previous seasons.

2.4.9. Conclusions on the clinical efficacy

The pivotal study MI-CP208 and the supportive adult study MI-CP185 were well conducted and analyses performed as planned. Both studies met their primary endpoints, demonstrating that the immunogenicity of the quadrivalent formulation was non-inferior to the immunogenicity of the trivalent comparators. The analyses of GMFR ratios further support the conclusion of non-inferiority.

Based on the evidence provided, it can be concluded that overall the potential immune interference caused by adding a fourth strain to the vaccine formulation is considered unlikely. This conclusion is especially supported by clinical data from the development program of the Applicant's bivalent and trivalent LAIVs as well as nonclinical data from the Q/LAIV development program, which overall indicate that clinically meaningful interference is not occurring.

Moreover, the CHMP considers that to further characterise the efficacy profile of this product, the Applicant should conduct a post-authorisation effectiveness study as indicated in the RMP.

2.5. Clinical safety

Patient exposure

Consistent with the bridging strategy pursued by the Applicant, the safety profile of Fluenz Tetra (or Q/LAIV) was directly compared to that of Fluenz (FluMist) in 2 pivotal studies: study MI-CP208 in paediatric subjects (2 to 17 years of age) and study MI-CP185 in adult subjects (18 to 49 years of age). In the additional clinical study MI-CP206 in adults (18-49 years of age), Q/LAIV was administered as a liquid stream to a single nostril using a novel blow-fill-seal (BFS) delivery system, which differs from the nasal spray used in studies MI-CP208 and MI-CP185 and in the approved FluMist. Because of this difference in vaccine delivery, this study MI-CP206 is considered supportive of Q/LAIV safety for AEs and SAEs only, and not for solicited symptoms. In order to examine a larger safety database for the occurrence of rare events, a pooled analysis of safety was performed for AE and SAE data from studies MI-CP185 and MI-CP206. Since the safety of Q/LAIV was compared to that of FluMist in all studies, there is no placebo or control group to estimate the background rate of safety events, and therefore the rate difference between subjects who received Q/LAIV and those who received FluMist is the most relevant assessment.

A total of 3779 subjects received at least one dose of Q/LAIV in the 3 studies that contribute to the Q/LAIV safety assessment, with 1382 of these subjects belonging to the target population of paediatric subjects, aged 2 to 17 years. The paediatric study provided over 98% confidence to detect an AE occurring at a rate of 0.3% (1 in 330); the 2 adult studies combined provided over 99% confidence to detect an AE occurring at a rate of 0.2% (1 in 500). All studies combined provided over 99% confidence to detect an AE occurring at a rate of 0.13% (1 in 770). Data are somewhat limited for the adolescent subgroup of the target population (299 subjects exposed in the age group 9 to 17 years). The CHMP however agrees with the Applicant that the safety data generated in adults and in younger children can provide reassurance regarding the safety of Q/LAIV in adolescents. The number of subjects exposed to Fluenz Tetra is considered acceptable for the bridging strategy that is pursued by the Applicant.

Adverse events

Solicited symptoms, which were actively queried from days 0 to 14 after dosing, were very commonly reported by subjects in both the Q/LAIV arm and the All FluMist arm in Q/LAIV studies: almost 50% of paediatric subjects (Q/LAIV recipients: 47.9%; FluMist recipients: 47.4%) and around 60% of adult subjects (Q/LAIV: 59.6%; FluMist: 60.0%) reported at least one solicited symptom in study MI-CP208 and study MI-CP185 respectively. The observed solicited symptom profile for Q/LAIV was similar to that observed after FluMist administration. The most notable difference in solicited symptoms occurred for fever, which was significantly more commonly observed in children who received Q/LAIV than those who received FluMist: fever occurred in 6.6% of Q/LAIV recipients versus 4.2% of FluMist recipients in the subset of subjects 2 to 8 years of age (p=0.036) (study MI-CP208, 2 dose group post dose 1). This difference is also reflected in the AE of pyrexia in paediatric subjects (study MI-CP208), which was more commonly reported by subjects in the Q/LAIV arm (1.7% in all subjects Q/LAIV post dose 1;

1.9% in the subgroup 2-8 years Q/LAIV post dose 1) than in the FluMist group (0.7% in all subjects FluMist post dose 1; 0.8% in the subgroup 2-8 years of age FluMist post dose 1). The increased rate of fever/pyrexia in paediatric subjects receiving Q/LAIV seems to be largely attributable to the increased rate in the 2 to 8 years age group, since less subjects 9 to 17 years of age receiving Q/LAIV reported fever as a solicited symptom (8/299; 2.7%) than did those receiving FluMist (6/204; 2.9%), and the rate difference for the AE of pyrexia was lower in this age group (2/299 or 0.7% in the Q/LAIV arm, 0/204, 0.0% in the All FluMist arm). Given that the overall rates of fever were low, fevers were generally mild and of short duration, high grade fever (≥ 39.5° C) was uncommon and occurred with comparable rates of in the Q/LAIV and FluMist group, and no febrile seizures were observed, the CHMP agrees with the Applicant that no significant impact on the overall tolerability of Q/LAIV compared to FluMist is to be expected as a consequence of this observed increase in fever after Q//LIAV dosing. Fever is included as a common adverse reaction in the Fluenz Tetra SmPC.

A number of imbalances between the Q/LAIV arm and the FluMist group were reported in the subset of subjects 9 to 17 years of age: the solicited symptom headache was reported by 2.8 percentage points more subjects in the Q/LAIV arm than in the All FluMist group, and proportionally more subjects reported ≥ 1 AE after Q/LAIV (23.4%) than after FluMist (12.7%), with the AE oropharyngeal pain being most disproportionally reported by subjects in this age group (5/299 or 1.7% of Q/LAIV subjects, no subjects who received FluMist). It should be noted that these observations were only based on a small sample size (299 subjects in the Q/LAIV arm, 204 subjects in the All FluMist).

The solicited symptoms reported reflect the mechanism of action of LAIVs and are consistent with those previously observed in studies with FluMist. There is no placebo or control group in the Q/LAIV studies to estimate the background rate of these events, but these solicited symptoms were also commonly reported in placebo recipients in placebo-controlled FluMist studies. Using the pooled analysis of FluMist safety in paediatric subjects 2 to 17 years of age to describe solicited symptoms occurring after FluMist dosing in controlled studies, the most important solicited symptoms by rate difference were runny/stuffy nose, which occurred at an 11.8 percentage point higher rate in FluMist recipients than TIV recipients and at a 6.8 percentage point higher rate in FluMist recipients than placebo recipients; and headache, which occurred at a 6.9 percentage point higher rate in FluMist recipients than placebo recipients, but only a 1.5 percentage point higher rate in FluMist recipients than TIV recipients. These solicited symptoms were not imbalanced when Q/LAIV was compared to FluMist; therefore, the addition of a fourth strain to FluMist to construct the Q/LAIV vaccine did not affect the rate of these solicited symptoms that are associated with FluMist dosing.

In general, adverse event were reported at similar rates by Q/LAIV and FluMist recipients, or with higher rates in the All FluMist group. In children and adolescents enrolled in the paediatric study MI-208, the preferred term AE reported with the highest rate difference was pyrexia (1.7% in the Q/LAIV arm; 0.7% in the All FluMist arm), which confirms the pattern that was seen for "fever" as a solicited symptom. Other preferred term events with a rate difference (Q/LAIV minus FluMist) of ≥ 0.5 percentage points were headache, oropharyngeal pain, epistaxis, abdominal pain, and nausea, but the proportion of subjects reporting these events was low (< 1%), and rate differences were small. Vomiting was the most frequently reported preferred term AE in both the Q/LAIV arm (2.6%) and the All FluMist group (2.2%). There were few events of wheezing occurring in either the Q/LAIV arm or the FluMist group; events were not imbalanced, and they were not clustered in the youngest subjects. In the pooled analysis of Q/LAIV safety in subjects 18 to 49 years of age, which combined data from both adult studies, one or more AEs were reported at a higher rate in the All FluMist group (17.3%) than in the All Q/LAIV group (16.6%). No preferred term AEs were reported with a rate difference of at least 0.5 percentage points. Cough and sneezing were the preferred term AEs reported by the largest proportion of subjects who received Q/LAIV (1.3% compared to 0.9% of FluMist subjects for both events).

Serious adverse event / deaths / other significant events

The rates of SAEs were low, particularly in children, and were balanced between Q/LAIV and FluMist recipients in the 2 pivotal Q/LAIV studies but not in the supportive Q/LAIV-BSF study where SAE were reported at a higher rate in the Q/LAIV-BSF group than in the FluMist group. The fact that the SAE rate was unexpectedly low in the FluMist group in this study could however be responsible for this imbalance.

Serious adverse events related to vaccination were only experienced by a very low number of subjects (2): 1 hypersensitivity event in a FluMist recipient and 1 spontaneous abortion in a Q/LAIV-BSF recipient. Hypersensitivity, which is a potential adverse event for all vaccines, has been previously reported for FluMist, and in the Fluenz/FluMist SmPC (as well as in the proposed SmPC for Fluenz Tetra) the contraindications include “Hypersensitivity to the active substances or to any of the excipients”. The term “hypersensitivity reactions” (including facial oedema, urticaria and very rare anaphylactic reactions) is also listed as an uncommon adverse reaction in this SmPC. For the SAE spontaneous abortion, the temporal association led to the assessment of a possible relationship to Q/LAIV, but according to the Applicant there is no overall pattern to suggest a causal relationship between Q/LAIV and spontaneous abortion. It can be argued however that the study population is too small for such a pattern to emerge, especially since a negative pregnancy test as well as the use of appropriate contraception are explicitly defined in the in-/exclusion criteria of the Q/LAIV studies. No definite conclusions on a putative causal relation between Q/LAIV and spontaneous abortion can therefore be drawn. In general, the use of live attenuated vaccines is not indicated in pregnant women. This is adequately pointed out in the SmPC, which explicitly states that Fluenz Tetra is not recommended during pregnancy.

Both in children enrolled in Study MI-CP208 as in adults enrolled in Studies MI-CP185 and MI-CP206, no NOCDs were considered related to study dosing, and there was no pattern of NOCDs that suggested an association with Q/LAIV dosing.

The two deaths that were reported in Q/LAIV studies both occurred in Q/LAIV-BSF recipients in supportive study MI-CP206. Both occurred in subjects with pre-existing medical illness and were considered unrelated to investigational product.

Laboratory findings

Clinical laboratory evaluations other than pregnancy testing prior to dosing were not required in the protocol for any Q/LAIV study. Laboratory evaluations performed for clinical indications were to be reported as an AE, SAE, and/or NOCD as appropriate.

Safety in special populations

With regards to special populations, the safety of Q/LAIV has not been assessed in subjects with asthma, wheezing or respiratory disease, or in immunosuppressed subjects. The CHMP agrees with the Applicant’s approach to apply the same restrictions for Q/LAIV as for the trivalent LAIV Fluenz.

As it was the case for Fluenz, Fluenz Tetra is not proposed for use in subjects < 24 months of age. This is adequately indicated in the Fluenz Tetra SmPC. A Paediatric Investigational Plan for this influenza vaccine, including a waiver for infants and toddlers from birth to less than 2 years of age, has been granted a positive opinion.

There are limited data on the use of Q/LAIV or FluMist in pregnant women, and the majority of data is from dosing with FluMist. Even though the existing data do not suggest an adverse effect of Q/LAIV or FluMist on maternal or foetal outcomes, there are inadequate data to assure the safety of Q/LAIV during pregnancy. Fluenz is not recommended during pregnancy and lactation, and this should also be the case for Fluenz Tetra. This is adequately reflected in the Fluenz Tetra SmPC.

Safety related to drug-drug interactions and other interactions

No studies of potential drug-drug or drug-food interactions were conducted with Q/LAIV. Because of the route of administration and the mechanism of action, potential interactions with food would not be expected. Drug interaction data from FluMist studies would apply to dosing with Q/LAIV. Potential interactions between Q/LAIV or Fluenz and other drugs or vaccines are summarized below.

- The concurrent use of Q/LAIV or Fluenz with antiviral agents that are active against influenza A and/or B viruses has not been evaluated; however, based upon the potential for antiviral agents with activity against influenza to reduce the effectiveness of Q/LAIV, Q/LAIV should not be administered until 48 hours after the cessation of such antiviral therapy, and these antiviral agents should not be administered until 2 weeks after administration of Q/LAIV unless medically indicated. If antiviral agents and Q/LAIV are administered concomitantly, revaccination should be considered when appropriate.
- Although there are no data linking Q/LAIV or Fluenz with Reye's syndrome, because of the association of Reye's syndrome with aspirin and wild-type influenza infection, Q/LAIV should not be administered to children and adolescents (2 to 17 years of age) who are receiving aspirin, salicylates, or aspirin containing therapy.
- The safety and immunogenicity Q/LAIV when administered concurrently with inactivated vaccines have not been determined. In the USA, where FluMist has been commercially available since 2003, the recommended practice as advised by the United States Advisory Committee on Immunization Practices (US ACIP) has been to consider that FluMist and inactivated vaccines "can be administered simultaneously or at any interval between doses" (CDC, 2006). This recommendation remains in the US ACIP's 2011 recommendation: "Any inactivated vaccine can be administered either simultaneously or at any time before or after a. . . live vaccine." (CDC, 2011)
- Q/LAIV can be administered concurrently with the following live, attenuated vaccines: measles, mumps, and rubella vaccine (MMR), varicella vaccine, and orally-administered polio vaccine (OPV) based on data from Studies D153-P522, AV018, and D153-P511, conducted with FluMist. These studies were previously submitted to the EMA and assessed. Concomitant administration of these vaccines did not change their safety profile.

Discontinuation due to adverse events

Withdrawals due to AEs could only be collected in the two-dose group in study MI-CP208, since the subjects in Studies MI-CP185 and MI-CP206 were to receive only one single dose of investigational product, and all subjects were to be followed for safety events for 180 days post last dose.

Discontinuation from dosing due to an AE occurred in 2 children enrolled in Study MI-CP208. These subjects were withdrawn from dosing by a parent /legal guardian (not by the investigator) due to AEs of vomiting and dizziness (one child who received Q/LAIV), and gastroenteritis (one child who received FluMist). These events were not considered by the investigator to be related to study dosing nor did they require termination of dosing in the investigator's judgment.

Post-marketing experience

In the extensive post-marketing experience of the trivalent LAIV FluMist (over 50 million doses distributed, primarily in the US), four distinct serious spontaneous reports of narcolepsy (with or without cataplexy) were identified. These cases occurred in children aged 36 months to 7 years, with an onset post FluMist vaccination of 1 to 6 months. Concern has been raised over a putative link between narcolepsy and H1N1 influenza vaccination following reports of narcolepsy in northern Europe after H1N1 vaccination. A retrospective cohort study by the National Institute for Health and Welfare in

Finland found a 12.7 fold increased risk of narcolepsy in Finnish children aged 4 to 19 years within 8 months of H1N1 vaccination with Pandemrix as compared to unvaccinated individuals in the same age group (Nohynek et al., PLoS ONE 2012). A correlation of narcolepsy onset with seasonal and annual patterns of upper airway infections, including H1N1 influenza, has been observed in China, with a 3-fold increase in narcolepsy onset following the 2009 H1N1 winter influenza pandemic (Han et al., Annals of Neurology 2011). No cases of narcolepsy with or without cataplexy were reported in the clinical trials for Q/LAIV or FluMist, and given the paucity of spontaneous post-marketing reports in association with FluMist (with observed reporting rate/expected incidence rate ratio of about 0.055) the current evidence does not support an association with FluMist. Narcolepsy is classified as an important potential risk for FluMist, and it is currently included as a potential risk in the Fluenz Tetra risk management plan.

2.5.1. Conclusions on the clinical safety

In summary, the safety profile of Fluenz Tetra was similar to that of the currently authorized Fluenz. The most notable difference in solicited symptoms occurred for fever, which was significantly more commonly observed in children who received Fluenz Tetra than those who received Fluenz; a difference also reflected in the AE of pyrexia. However, the overall rates of fever were low, high fevers (≥ 39.5°C) were uncommon, no febrile seizures were observed, and fevers were of short duration. This small increase in fever after Fluenz Tetra dosing is not expected to significantly alter the tolerability of the vaccine.

Adverse events and SAEs were proportionally similar between Fluenz Tetra and Fluenz; the events other than pyrexia disproportionately reported by Fluenz Tetra subjects, i.e. headache and oropharyngeal pain, occurred in < 1% of subjects, occurred late after dosing.

The overall similarity of solicited symptoms and AEs between Fluenz Tetra and Fluenz permits the understanding of the safety of Fluenz derived from its very large safety database to apply to the quadrivalent formulation of the vaccine. From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Fluenz Tetra SmPC. The recommendations and contraindications that currently apply to Fluenz are also valid for Fluenz Tetra. Most importantly, Fluenz Tetra will not be indicated for use in infants and toddlers under the age of 24 months. The use of Fluenz Tetra (and Fluenz) is not recommended in individuals with severe asthma and wheezing, and in pregnancy or breastfeeding, as there are limited data on the safety in these special populations.

2.6. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the Applicant fulfils the legislative requirements.

2.7. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 5.0, the PRAC considers by consensus that the risk management system for influenza vaccine (live attenuated, nasal) (Fluenz and Fluenz Tetra) in the prophylaxis of influenza in children and adolescents 24 months to less than 18 years of age is acceptable.

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

The Applicant identified the following safety concerns in the RMP:

Table 5: Summary of the Safety Concerns

Important identified risks	Medically significant wheezing in children under the age of 24 months Hypersensitivity (including anaphylaxis)
Important potential risks	Guillain-Barré Syndrome Bell's Palsy Secondary transmission to severely immunocompromised patients Inadvertent administration to immunocompromised patients Seizures and convulsions Encephalitis Neuritis Vasculitis Vaccination Failure (Lack of Efficacy) Narcolepsy with or without cataplexy
Missing information	There is limited information regarding safety of Fluenz and Fluenz Tetra in the following populations: Children under the age of 24 months Elderly Pregnant/lactating women Severe asthmatics Immunocompromised vaccine recipients Individuals with severe chronic illness

The PRAC agreed.

- **Pharmacovigilance plans**

Table 6: Ongoing and planned studies in the PhV development plan

Study / Activity Type, Title and Category (1-3)	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (planned or actual)
MI-MA194: A Postmarketing Observational Evaluation of the Safety of Fluenz in Children and Adolescents with High-risk Conditions (to include Q/LAIV following approval) Observational, Category 3	To investigate the safety of Fluenz and Fluenz Tetra in high risk paediatric populations.	All cause Serious Adverse Events (SAEs), lower respiratory SAEs and other Medically Attended Events (MAEs) in patients receiving LAIV.	2013-2014 Influenza Season	First annual report submitted Oct2014
MA-VA-MEDI3250-1115 Postmarketing Safety Study of Q/LAIV in Subjects 2 Through 49 Years of Age (USA) Observational, Category 3	To compare MAEs rates in patients receiving Q/LAIV, TIV or no vaccine	MAEs in patients receiving Q/LAIV	2013-2014 Influenza Season	First annual report submitted Jul2016
MA-VA-MEDI3250-1116 A Case Control Study of the Effectiveness of Q/LAIV Versus Inactivated Influenza Vaccine and No Vaccine in Subjects 2-17 Years of Age (USA) Case-Control, Category 3	To evaluate the effectiveness of Q/LAIV vaccination in children 2 through 17 years of age	Potential Risk: Lack of effect	2013-2014 Influenza Season	First annual report submitted Sep2014

SAE= Serious Adverse Event MAE= Medically Attended Event

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The PRAC also considered that routine PhV is sufficient to monitor the effectiveness of the risk minimisation measures.

- **Risk minimisation measures**

Table 7: Summary table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Medically significant wheezing in children less than 24 months of age	<PRODUCT> is not indicated in children less than 24 months of age. This information is included in the "Posology and method of administration," "Special warnings and precautions for use" and "Undesirable effects" sections of the approved FLUENZ (SmPC Sections 4.2, 4.4 and 4.8) and proposed Q/LAIV SmPC (SmPC Sections 4.2 and 4.8). Information is also included in the <PRODUCT> Package Leaflet.	None applicable, routine risk minimisation activities are sufficient.
Hypersensitivity (including anaphylaxis)	This is a class effect that is included in the "Contraindications" section, and is listed in the "Undesirable effects" section of the approved FLUENZ and proposed Q/LAIV product SmPC (Sections 4.3 and 4.8) and also included in the Package Leaflet. The following statement is also in the "Special warnings and precautions for use" section of the approved FLUENZ and proposed Q/LAIV SmPC: "As with most vaccines, appropriate medical treatment and supervision should always be readily available in case of an anaphylactic event following the administration of <PRODUCT>." The wording in the proposed Q/LAIV SmPC is as follows: "As with most vaccines, appropriate medical treatment and supervision should always be readily available to manage an anaphylactic event or serious hypersensitivity event following the administration of <PRODUCT>."	None applicable, routine risk minimisation activities are sufficient.
Guillain-Barré syndrome	This is listed as one of the adverse reactions that have been reported post-vaccination in the post-marketing setting in the "Undesirable Effects" section of the approved FLUENZ and proposed Q/LAIV SmPC (SmPC Section 4.8).	None applicable, routine risk minimisation activities are sufficient.
Bell's palsy	Bell's palsy is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Secondary transmission to severely immunocompromised patients	Information is included in the "Special warnings and precautions for use" section of the approved FLUENZ and proposed Q/LAIV SmPC (SmPC Section 4.4) and also included in the Package Leaflet.	None applicable, routine risk minimisation activities are sufficient.
Inadvertent administration to immunocompromised patients	Information is included in the "Contraindications" section of the approved FLUENZ and approved Q/LAIV SmPC (SmPC Section 4.3) and also included in the Package Leaflet.	None applicable, routine risk minimisation activities are sufficient.
Seizures and convulsions	Seizures and convulsions are included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Encephalitis	Encephalitis is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Neuritis	Neuritis is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Vasculitis	Vasculitis is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Vaccination failure (lack of efficacy)	Section 2 of the approved FLUENZ and proposed Q/LAIV SmPC ("Qualitative and Quantitative Composition") will provide details of the official viral strains included in each seasonal product. Section 5.1 of the approved FLUENZ and proposed Q/LAIV SmPC ("Pharmacodynamic properties") provides information on the demonstrated efficacy of <PRODUCT> from controlled clinical studies. Efficacy was not demonstrated to be 100% therefore it should be understood that the vaccine may not be effective in all recipients.	None applicable, routine risk minimisation activities are sufficient.
Narcolepsy with or without cataplexy	Narcolepsy with or without cataplexy is included in this RMP as a potential risk, therefore, no risk minimisation activities are deemed necessary until such time it may be confirmed as an identified risk.	None applicable
Children under the age of 24 months	<PRODUCT> is not indicated for use in children younger than 24 months (approved FLUENZ and proposed Q/LAIV SmPC Section 4.1, Therapeutic indications). The "Posology and method of administration section" of the proposed Q/LAIV SmPC (Section 4.2) contains the following instructions: "Fluenz Tetra should not be used in infants and toddlers below 24 months of age because of safety concerns regarding increased rates of hospitalisation and wheezing in this population (see section 4.8)." The "Special warnings and precautions for use" section of the approved FLUENZ (Section 4.4) contains the following instructions: "Do not administer <PRODUCT> to infants and toddlers younger than 12 months." "It is not recommended to administer <PRODUCT> to infants and toddlers 12-23 months of age." The "Undesirable effects" section of the approved FLUENZ and proposed Q/LAIV SmPC (Section 4.8) contains the following statement: "<PRODUCT> is not indicated for use in infants and toddlers younger than 24 months (see section 4.4)."	None applicable, routine risk minimisation activities are sufficient
Elderly	<Product> is not indicated for use in elderly adults (approved FLUENZ and proposed Q/LAIV SmPC Section 4.1, Therapeutic indications).	None applicable
Pregnant and breast-feeding women	Pregnancy and lactation-related information is found in the "Fertility, pregnancy and lactation" section of the approved FLUENZ and proposed Q/LAIV SmPC (Section 4.6) and the Package Leaflet. <PRODUCT> is not recommended for use in women who are pregnant or during breast-feeding.	None applicable, routine risk minimisation activities are sufficient
Severe asthmatics	Information related to severe asthmatics is found in the "Special warnings and precautions for use section" of the approved FLUENZ and proposed Q/LAIV SmPC (section 4.4) and the Package Leaflet. <PRODUCT> is not recommended for use in patients with severe asthma or active wheezing. <PRODUCT> has not been adequately studied in children and adolescents with severe asthma or active wheezing in clinical studies.	None applicable, routine risk minimisation activities are sufficient

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Immunocompromised vaccine recipients	Information related to clinically immunocompromised patients is found in the "Contraindications" section in the approved FLUENZ and proposed Q/LAIV SmPC (Section 4.3) and in the Package Leaflet. <PRODUCT> is contraindicated in children and adolescents who are clinically immunodeficient due to conditions or immunosuppressive therapy such as: acute and chronic leukaemias; lymphoma; symptomatic HIV infection; cellular immune deficiencies; high-dose corticosteroids. <PRODUCT> is not contraindicated for use in people with asymptomatic HIV infection; in people who are receiving topical/inhaled corticosteroids, low-dose systemic corticosteroids or those receiving corticosteroids as replacement therapy, e.g. for adrenal insufficiency.	None applicable, routine risk minimisation activities are sufficient
Serious chronic disease	There is currently no evidence to suggest that vaccine recipients with serious chronic diseases (excluding those who are clinically immunodeficient, see above) are at increased risk compared to the general population. Therefore, no action is deemed necessary.	None applicable

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

The CHMP endorsed this advice without changes.

2.8. User consultation

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to the trivalent vaccine. The bridging report submitted by the Applicant has been found acceptable.

3. Benefit-Risk Balance

Benefits

Beneficial effects

First of all LAIVs provide a needle-free alternative to intramuscular vaccination, secondly the development of a quadrivalent influenza vaccine represents in principle an advance over currently available trivalent influenza vaccines, as it expands the number of protective strains included in the vaccine, thereby increasing the likelihood of protection against circulating B strains of influenza. Providing protection that is as broad as possible may be of particular importance for children and adolescents. Although influenza B causes disease in all age groups, its incidence relative to influenza A appears to be highest among older children and young adults. Furthermore, while influenza B causes mortality in all age groups, it appears to be a disproportionate cause of pediatric influenza deaths.

Concerning study results for Fluenz Tetra, the pivotal paediatric study MI-CP208 showed that immune responses to Q/LAIV were non-inferior to Fluenz since the upper bound for each of the four 95% CIs for the GMT ratios (Fluenz divided by Q/LAIV) was $\leq$ 1.5, which was a pre-specified and acceptable target. The GMT ratios ranged between 1.04 and 1.21. The highest value for the upper bound of the 95% CI was 1.37 for the B/Yamagata strain.

The supportive adult study MI-CP185 showed that immune responses to Q/LAIV were non-inferior to Fluenz since the upper bound for each of the four 95% CIs for the GMT ratios (Fluenz divided by Q/LAIV) was ≤ 1.5. The GMT ratios ranged between 0.92 and 1.10. The highest value for the upper bound of the 95% CI was 1.25 for the B/Yamagata strain.

Hence, both studies met their primary endpoints, demonstrating that the immunogenicity of the quadrivalent formulation was non-inferior to the immunogenicity of the trivalent comparators based on HAI, NAI and MN assays. The immune response to vaccination with each of the influenza vaccine strains contained in Q/LAIV was not inferior to that occurring after vaccination with the same strains contained in the licensed (trivalent) Fluenz vaccine based on these assays.

Efficacy studies with the trivalent Fluenz vaccine in young subjects showed that receipt of Fluenz in Year 1 was associated with statistically significant efficacy against culture-positive influenza illness and such effect persisted in part into the subsequent season

Uncertainty in the knowledge about beneficial effects

The efficacy of LAIV in children above the age of 8 has been questioned. A recent comprehensive review of influenza vaccines states that trivalent LAIVs have consistently shown highest efficacy in young children (from 6 months to 7 years old), whereas this is not always the case for individuals from 8 to 59 years of age (Osterholm et al., 2012).

The non-inferiority immunogenicity studies did not address culture-confirmed infection cases but instead are based upon serological assays which provide an indirect measure of efficacy in lack of an established correlate of protection. There remain therefore uncertainties to the extent non-inferiority established on the basis of humoral immunity corresponds to clinical non-inferiority.

The immunogenicity data (HAI, NAI and MN assays) show that the immune responses obtained with Fluenz Tetra vs. Fluenz were similar but lower than expected with regard to the A/H1N1 and A/H3N2 strains, whose antibody titres were not much higher than baseline values.

The efficacy of the vaccine following repeated yearly revaccinations would benefit from further evidence.

Overall these uncertainties are expected to be addressed in the post-authorisation effectiveness study described in the RMP.

Risks

Unfavourable effects

The safety profile of Fluenz Tetra is similar to that of the authorized Fluenz.

The most notable difference in solicited symptoms relates to fever, whose incidence was significantly higher in children who received Fluenz Tetra vs. Fluenz (respectively 6.6% versus 4.2% in the subset 2 to 8 years of age (p=0.036) in study MI-CP208, 2 dose group post dose 1). In addition, the variable incidence of influenza B infection across seasons would render the inclusion of both B strains redundant in seasons with low incidence, whereas potential side effects such as increased fever associated with the exposure of subjects to the two influenza B strains remain. In such seasons, the benefit of adding a second B strain may thus become uncertain. However, the overall rates of fever remained low, fevers were generally mild and of short duration. High fevers (≥ 39.5°C) were uncommon and occurred with comparable rates between vaccines. No febrile seizures were observed. This limited increase in fever after Fluenz Tetra vaccination is not expected to significantly alter the tolerability of the vaccine. Fever is included as a common adverse reaction in the Fluenz Tetra Product Information.

Adverse events and SAEs were proportionally very similar between Fluenz Tetra and Fluenz. The other events disproportionately reported by Fluenz Tetra subjects, i.e. headache and oropharyngeal pain, occurred in < 1% of subjects and late after dosing.

The overall similarity of solicited symptoms and AEs between Fluenz Tetra and Fluenz allows for the extrapolation of the safety profile from Fluenz to the quadrivalent formulation Fluenz Tetra.

The recommendations and contraindications that currently apply to Fluenz do also apply to Fluenz Tetra. Specifically, Fluenz Tetra should not be indicated for use in infants and toddlers under the age of 24 months.

Uncertainty in the knowledge about the unfavourable effects

The use of Fluenz Tetra (and Fluenz) is not recommended in individuals with severe asthma and wheezing, neither during pregnancy or breastfeeding, as there are limited safety data in these special populations.

Whether repeated use of the vaccine is equally well tolerated over time is not yet demonstrated. It cannot be excluded that allergic reactions may occur after repeated use.

It is currently uncertain whether the incidence of serious adverse events with very low background rates (i.e., GBS, Bell's palsy, and narcolepsy) can be determined through the proposed post-authorization safety studies (MI-MA194, MI-MA1115). However the ten years long marketing experience in the US has not raised major concerns for the time being.

Benefit-risk balance

Importance of favourable and unfavourable effects

The LAIV vaccine provides a needle-free alternative to injection for influenza vaccination programmes in children and Q/LAIV increases vs. T/LAIV the likelihood of protection against both influenza B lineages.

The efficacy of Fluenz Tetra in children can be extrapolated from its trivalent vaccine counterpart, based on demonstrated non-inferiority versus Fluenz in a predefined immunogenicity bridging strategy with similar results across different tests. Additional clinical and nonclinical evidence further support the bridging approach, despite a low humoral response to A/H1N1 and A/H3N2 by HAI in the 2 to < 18 years age group.

The safety profile of Fluenz Tetra is similar to that of the authorized Fluenz and is therefore acceptable. The recommendations and contraindications that currently apply to Fluenz do also apply to Fluenz Tetra. Specifically, Fluenz Tetra should not be indicated for use infants and toddlers under the age of 24 months.

Benefit-risk balance

Overall the benefit/risk balance for Fluenz Tetra is positive.

Discussion on the benefit-risk balance

Efficacy of Q/LAIV in the prevention of influenza infection is considered sufficiently studied despite some remaining uncertainties as detailed in sections above. For instance, efficacy upon repeated vaccinations is not yet fully ascertained.

The safety profile of Fluenz Tetra is acceptable, although expectedly not fully characterized yet in respect to long-term safety data. Post-marketing safety effectiveness studies have been agreed to address such uncertainties and are detailed in the RMP.

The proposed test-negative, case-control effectiveness study (MI-MA1116) is anticipated to substantiate and confirm efficacy.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Fluenz Tetra in the prophylaxis of influenza in children and adolescents from 24 months to less than 18 years of age is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal products subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European Medicines Agency web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

When the submission of a PSUR and the update of a RMP coincide, they should be submitted at the same time.

In addition, an updated RMP should be submitted:

At the request of the European Medicines Agency;

Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal

product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CHMP review of data, the CHMP considers that the B/Yamagata strain contained in Fluenz Tetra is to be qualified as a new active substance in itself.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan (P/0234/2012) and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.